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Drinking Water Criteria Document for Styrene

Syracuse Research Corp., NY

Prepared for:

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16. ABSTRACT

This document deals with the drinking water health effects of Styrene through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

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DRINKING WATER CRITERIA DOCUMENT FOR STYRENE

Prepared for

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or antici- pated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1984; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

AP	Alkaline phosphatase
BOD	Biological oxygen demand
CNS	Central nervous system
DNA	Deoxyribonucleic acid
DWEL	Drinking Water Equivalent Level
EEG	Electroencephalogram
GGT	Gamma glutamyltransferase
GI	Gastrointestinal
GSH	Reduced glutathione
HA	Health advisory
LDH	Lactic dehydrogenase
MAC	Maximum allowable concentration
MTD	Maximum tolerated dose
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NOAEL	No-observed-adverse-effect level
OCT	Ornithine carbamoyltransferase
RfD	Reference dose
RNA	Ribonucleic acid
SGOT	Serum glutamic oxalocetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TWA	Time-weighted average

I. SUMMARY

Styrene is a clear, colorless liquid with a characteristic odor that is caused by aldehydic impurities. Styrene has a molecular weight of 104.16, a vapor pressure at 20°C of 4.53 torr and a water solubility of 320 mg/ℓ. It is miscible in most organic solvents and has a log octanol/water partition coefficient of 2.95. Styrene is likely to volatilize from water with a half-life of a few days to weeks (based on an EXAMS model) and to biodegrade significantly. Chemical and photochemical processes are not important removal processes, with the possible exception of the chlorination of styrene during drinking water chlorination.

Pharmacokinetic studies have shown that styrene is readily absorbed from the GI tract of rats; absorption from the lungs of animals and humans is also rapid. Styrene is widely and rapidly distributed through the body with a tendency to sequester in adipose tissue. Elimination from tissues and organs of experimental mammals is virtually complete within 1 day, while half-lives of 2-4 days have been estimated for human adipose tissue. The major metabolic pathways involve the conversion of styrene to styrene oxide, a highly reactive intermediate, by hepatic drug-metabolizing enzymes. Styrene oxide, in turn, may be metabolized to styrene glycol, which is excreted in the urine as a glucuronide conjugate or as its metabolites mandelic, phenylglyoxylic, benzoic or hippuric acid. Direct human evidence indicated that styrene-7,8-oxide and styrene glycol can be measured in the blood. Detoxification of styrene oxide may also proceed by glutathione conjugation. Styrene is excreted almost completely (90-97%) as urinary metabolites in both rats and humans. In animals, elimination of styrene in expired air and urine is rapid and biphasic log-linear at low exposure levels but displays saturation kinetics at high exposures (e.g., 600 ppm inhalation, 500 mg/kg oral). Biphasic elimination has also been observed in humans with urinary mandelic acid alpha and beta half-lives of ~4 and 25 hours, respectively.

The acute toxicity of styrene is relatively low with an oral LD_{50} for rats reported as ~5000 mg/kg and LC_{50} values ranging from 2400-4900 for 4- to 6-hour exposures of rats and mice. General signs of toxicity following repeated nonlethal exposures of animals include reduced weight gain, increased kidney and liver weights, and lung congestion. An increase in mixed-function oxidase activity and a decrease in glutathione-S-transferase activity in rat liver have been reported following oral administration of styrene for 7 or ~100 days. Minor hepatotoxic effects of oral exposure to styrene have been indicated in both dogs and rats. A dose of 400 mg/kg/day, 6 days/week for 100 days resulted in focal necrosis in rat liver and elevated levels of SGOT and SGPT. In the brain, repeated oral doses of styrene (~900 mg/kg) resulted in elevated levels of noradrenaline and serotonin and decreased activity of monoamine oxidase in rats, while increased sensitivity of dopamine receptors was observed following single or repeated oral doses of $\geq$200 mg/kg. Results of inhalation studies of styrene in rats support the oral exposure results. Alterations in hepatic enzyme activity, biochemical changes in the brain and a transient increase in the motor conduction velocity of the tail nerve have been reported following repeated inhalation exposures.

Styrene was not mutagenic in *Salmonella typhimurium* in the absence of a mammalian metabolic activation system; there were mixed positive and negative results in the presence of metabolic activation. Styrene oxide was mutagenic in *S. typhimurium* with and without metabolic activation. Styrene has been shown to be mutagenic in yeast, fruit flies and cultured mammalian cells with metabolic activation. Styrene produced an increased incidence of chromosomal aberrations in cultured mammalian cells, isolated human lymphocytes, and when tested *in vivo* in rats and mice.

No teratogenic effects of styrene have been observed in rats using the oral route or in rats, mice, rabbits and hamsters following inhalation exposure. Fetotoxicity was reported in mice exposed to 250 ppm styrene, 6 hours/day on days 6-16 of gestation and in hamsters exposed to 1000 ppm styrene, 6 hours/day on days 6-18 of gestation.

In controlled experiments with human volunteers, single inhalation exposures to 376 ppm of styrene resulted in eye and throat irritation, altered neurophysiological function and increased reaction time. CNS effects of occupational exposure to styrene including increased reaction time, decreased visuomotor and psychomotor performance, increased incidences of abnormal EEGs and mild sensory neuropathy have been documented. An estimated threshold exposure for decreased visuomotor accuracy is ~36 ppm for an 8-hour TWA, while a marked change in the proportion of abnormal EEGs occurred at ~31 ppm TWA. Equivocal results have been reported regarding elevation of SGOT, SGPT and GGT in workers exposed to styrene, while reports of abnormal respiratory function and minimal hematologic and renal effects have yet to be verified. An increase in chromosomal damage in peripheral lymphocytes has been reported in workers with exposure to styrene.

A reported association between spontaneous abortions and exposure to styrene in Finnish chemical workers is difficult to interpret because of the small sample size, unquantified exposure levels and possible confounding effects of multiple chemical exposures. Spontaneous abortion rates were not elevated in 67 workers exposed to ~66 ppm styrene.

Styrene has been tested for carcinogenicity in several animal bioassays. The animal data suggest that styrene may be a human carcinogen; however, these data are currently undergoing further Agency analysis. An increased incidence of lung tumors was found in male B6C3F1 mice given oral doses of 92 and 184 mg/kg/day for 78 weeks. In another study a statistically significant increased incidence of liver tumors was observed in C57B1 mice administered styrene orally at 42.9 mg/kg/day. In the same study negative results were reported in BDIV rats and O_{20} mice. A statistically significant increased incidence of lung tumors was observed in O_{20} mice; however, there was an extremely high background tumor rate in this strain. Increased incidence of mammary adenocarcinomas in female Sprague-Dawley rats provides evidence of styrene carcinogenicity from inhalation.

Epidemiological studies provide insufficient evidence to evaluate the human carcinogenic potential for styrene. Most of the available studies are limited because of multiple chemical exposures and/or small cohort sizes. There is evidence suggesting that there is an elevated risk for neoplasms of the lymphatic and hematopoietic tissues in workers in the styrene-butadiene rubber industry and the styrene-polystyrene manufacturing industry, but this risk has not been confirmed nor has styrene been indicated as the causative agent.

Several investigators have observed covalent binding of styrene and styrene oxide to cellular macromolecules, which is a suggestive mechanism of toxicity. An *in vitro* study has demonstrated the interaction of styrene and styrene oxide with DNA. Mechanisms for the neurotoxic effects of styrene exposure have not yet been elucidated. Reported biochemical changes in the brain include increased serotonin and noradrenaline and increased sensitivity of dopamine receptors.

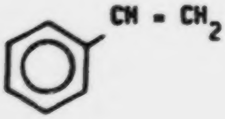
A NOAEL of 22.5 mg/kg/day was used to derive a 1-day HA for ingestion of styrene in drinking water of 22.5 mg/ℓ for a 10 kg child. No data were identified in the available literature that would be suitable for the derivation of a 10-day HA. It was therefore recommended that the longer-term HA of 20 mg/ℓ for a child be adopted as the 10-day HA. Longer-term HAs of 20 mg/ℓ for a 10 kg child and 70 mg/ℓ for a 70 kg adult were derived from a NOAEL of 200 mg/kg for hematological effects in dogs. A lifetime DWEL of 7.0 mg/ℓ was derived from a NOAEL of 200 mg/kg for hematological effects in dogs.

Collectively, three animal bioassays along with metabolic and genotoxicity studies provide sufficient evidence of styrene carcinogenicity in animal test systems. The weight-of-evidence classification for styrene is currently undergoing Agency review. This document will be revised in accordance with the outcome of this review.

II. PHYSICAL AND CHEMICAL PROPERTIES

Structure and Identification

The structure, molecular formula, molecular weight, Chemical Abstracts Service (CAS) Registry number, Registry of Toxic Effects of Chemical Substances (RTECS) number and common synonyms for styrene are listed below:



Molecular Formula: C_8H_8

Molecular Weight: 104.16

CAS Registry Number: 100-42-5

RTECS Number: WL3675000

Synonyms: vinyl benzene, cinnamene, phenylethylene, ethenylbenzene

Physical and Chemical Properties

Styrene is a clear, colorless liquid with a characteristic sweet and pleasant odor if pure, but usually contains aldehydes that have a typical penetrating smell, sharp and unpleasant (Verschueren, 1983). Styrene has a vapor pressure of 4.53 torr at 20°C and a water solubility of 320 mg/ℓ at 25°C. A more complete listing of the physical properties of styrene is provided in Table II-1.

Stability in Water

The dominant aquatic environmental removal processes for styrene are volatilization and biodegradation. Chemical and photochemical processes are likely to be less important; sorption to sediments and biota may occur. A possible important chemical reaction is the reaction of chlorine in water with styrene.

TABLE II-1

Physical Properties of Styrene*

Molecular Weight	104.16
Boiling Point (1 atm)	145°C
Melting Point	-30.6°C
Density (20°C)	30.86 g/cm³
Vapor Pressure (20°C) **(25°C)**	4.53 torr 6.18 torr
Water Solubility (25°C)	320 mg/ℓ
Lipid Solubility	miscible in acetone, carbon tetrachloride, benzene, n-heptane, ether and ethanol
Log Octanol/Water Partition Coefficient	2.95 (Hansch and Leo, 1979)
Adsorptive Capacity of Activated Carbon	120 mg/g (Ramanathan, 1984)
Conversion Factors	1 mg/m³ = 0.235 ppm 1 ppm = 4.26 mg/m³
Odor Threshold (water)	0.01 mg/ℓ (Amoore and Hautala, 1983)

*Source: Lewis et al., 1983, except where noted.

Styrene will volatilize from water relatively rapidly. The half-lives for removal, based on an EXAMS model (Burns et al., 1981), vary from 3 days for removal from a pond to ~13 days for removal from an oligotrophic lake. The input data used in the simulation was obtained from Lewis et al. (1983).

Bridie et al. (1979) used filtered sewage as an inoculum and found 42% degradation (based on theoretical oxygen demand) in a 5-day standard BOD test. After acclimation, the inoculum degraded 80% of the styrene in 5 days. Price et al. (1974) used settled domestic wastewater as a BOD inoculum and found 65% degradation in 5 days. Although none of the above utilized ambient waters as an inoculum, they nonetheless indicate styrene will be degraded relatively rapidly by microorganisms. In addition, Wilson et al. (1983b) reported slight degradation (2.3-12% degradation/week) in samples of groundwater uncontaminated with surface microbes.

Chemical and photochemical (styrene does not absorb light significantly above 290 nm) degradation in natural water systems is unlikely to occur at a significant rate. Based on the method described in Mabey et al. (1981), the calculated peroxy radical rate constant for addition to styrene is 24 M^{-1} hr^{-1}. The half-life calculated from this by the EXAMS model is several orders of magnitude slower than either biodegradation or volatilization. The reaction of chlorine in water with styrene to form the chlorohydrin is of significance during the chlorination of drinking water. Kinetic data for this reaction were not found in the available literature; however, it is known to be fast (March, 1968). Styrene has been detected in finished drinking water. Consequently, the reaction does not appear to be sufficiently fast so as to preclude the detection of styrene. This conclusion assumes the drinking waters tested by Shackelford and Keith (1976) were chlorinated.

Summary

Styrene is a clear, colorless liquid with a characteristic odor that is caused by aldehydic impurities (Lewis et al., 1983). Styrene has a molecular weight of 104.16, a vapor pressure at 20°C of 4.53 torr and a water solubility of 320 mg/ℓ. It is miscible in most organic solvents (Lewis et al., 1983) and has a log octanol/water partition coefficient of 2.95. Styrene is likely to volatilize from water with a half-life of a few days to weeks (based on an EXAMS model) and to biodegrade significantly (Price et al., 1974; Bridie et al., 1979; Wilson et al., 1983b). Chemical and photochemical processes are not important removal processes, with the possible exception of the chlorination of styrene during drinking water chlorination.

III. TOXICOKINETICS

Absorption

Available data regarding the absorption of styrene from the GI tract indicate that absorption is rapid and virtually complete.

Withey (1976) administered solutions of styrene by gastric intubation to rats fasted overnight. Administration of a total dose of 3.147 mg styrene in 10 mℓ aqueous solution resulted in a peak blood level of ~6 μg/mℓ within minutes following dosing. When styrene was admininstered in vegetable oil as a total dose of 32.61 mg in 2 mℓ, a peak blood level of ~12 μg/mℓ was not reached until ~100 minutes after dosing. The high lipid solubility of styrene was offered as a possible explanation for the slower uptake rate, with styrene remaining associated with the oil in the GI tract. The oil/blood partition coefficient is 130 (Van Rees, 1974).

Plotnick and Weigel (1979) located <10% (~77% of the total dose was recovered) of a 20 mg/kg dose of ^{14}C-styrene (uniform ring label) in the GI tract of rats 8 hours after oral intubation using a corn oil solution. Within 24 hours, ~90% of the dose had been excreted in the urine, while <2% was found in the feces.

When Sauerhoff et al. (1976) administered uniform ring-^{14}C-styrene (in corn oil solution) to rats, 95% of a 50 mg/kg dose was excreted in the urine within 72 hours, while 1% was expired and 4% was recovered in the feces. A 500 mg/kg dose was also recovered largely in the urine (90%), with an increased percentage expired (9%) and only 1.5% excreted in the feces.

Pertinent data regarding the absorption of styrene from the GI tract of humans were not located in the available literature.

The dermal penetration rate of styrene was measured _in vitro_ by applying 1 mℓ of styrene to 2.55 cm^2 of excised rat abdominal skin in a diffusion cell (Tsuruta, 1982). The penetration rate was estimated as 4.8 nmoles/min/cm^2 after a 1.67-hour lag time.

High styrene absorption by skin was demonstrated _in vivo_ by immersing tails of rats in styrene for 1 hour (Shugaev, 1969). Styrene concentrations of 14.2 mg/100 cc and 14.7 mg/100 cc were found in the brain and liver, respectively, following immersion.

Dutkiewcz and Tyras (1968) showed that there is significant absorption of styrene through skin in humans. When styrene was applied to the forearms of male subjects, absorption rates were 9-15 mg/cm^2/hr for liquid styrene and 40-180 μg/cm^2/hr for aqueous solutions of 66.5-269 mg/ℓ (66.5-269 ppm).

The uptake of styrene following inhalation exposure of rats is rapid (Withey, 1976; Withey and Collins, 1979; Ramsey and Young, 1978, 1980). Blood levels of styrene were measurable within minutes following exposure and continued to increase during the 5 hour exposure to levels of ~1.5, 40, 150 and 200 μg/mℓ for exposure concentrations of 50, 500, 1200 and 3000 ppm, respectively (Withey, 1976). Plateau levels of styrene in the blood of 0.8, 1.5, 25 and 64 μg/mℓ corresponding to concentrations of 80, 200, 600 and 1200 ppm, respectively, were reached generally within 6 hours during a 24-hour exposure study (Ramsey and Young, 1978, 1980).

Styrene uptake and absorption has been the subject of a number of human inhalation studies (Fiserova-Bergerova and Tersinger, 1965; Bardodej and Bardodejova, 1970; Fernandez and Caperos, 1977; Engstrom et al., 1978a,b; Teramoto and Horiguchi, 1979). The findings of these studies indicate that pulmonary retention of styrene is ~2/3 of the supplied concentration with considerable variation in measured uptake between individuals and studies (mean uptakes ranged from 59-89%).

Astrand et al. (1974) and Engstrom et al. (1978a) found that arterial levels of styrene in humans increase as a function of increased ventilation, which is due to exercise, and time of exposure. Astrand et al. (1974) found that alveolar concentrations reached a plateau within ~1 minute, which implies that absorption of styrene across the alveolar membrane into the blood is rapid. This rapid absorption is consistent with the fairly high blood/air partition coefficients of 18, 32 and 52 estimated for styrene by Lindqvist (1978), Van Rees (1974) and Sato and Nakajima (1979), respectively.

Direct human evidence from a recent report (Löf et al., 1986) indicated that styrene-7,8-oxide and styrene glycol can be measured in the blood. Ten healthy men occupationally exposed to styrene were studied during 3 consective workdays in two glass-fiber reinforced plastic factories. The mean external exposure level was 99 mg/m³. The styrene concentration in venous blood was only measured in subjects working in one plant. The concentration of styrene-7,8-oxide measured in most blood samples was 0.02 μmol/ℓ, which is at the detection limit. In one blood sample a somewhat higher concentration of styrene-7,8-oxide (0.04 μmol/ℓ) was measured.

Distribution

The distribution of styrene following oral administration was studied in rats given single doses of 20 mg/kg ^{14}C-styrene (uniform ring labelled) by corn oil gavage (Plotnick and Weigel, 1979). Peak tissue levels of styrene were reached within 2-4 hours. The organs with the highest concentrations were the kidney (46 µg/g in males; 25 µg/g in females), liver (13 µg/g in males; 7 µg/g in females) and pancreas (10 µg/g in males; 6 µg/g in females), with lower levels in lungs, heart, spleen, adrenals, brain, testes and ovaries. Suprarenal fat concentrations rose to 10 and 6 µg/g in males and females, respectively, 4 hours after dosing, then dropped to 2 µg/g at 12 hours. Styrene was also detected in muscle, bone marrow, and skin. By 24 hours, the concentration was <1 µg/g in all tissues examined. In EPA's Broad Scan Analysis of Human Adipose Tissue for Volatile Organics, styrene has been detected in every sample analyzed. Of the 17 volatile organics tested, styrene was consistently reported among the highest levels measured (U.S. EPA, 1986c).

Withey and Collins (1977) administered intravenous styrene to rats in doses of 1.337, 4.011, 6.695 and 9.359 mg/kg. The mean apparent volume of distribution (Vd) was ~220 mℓ, which is ~10 times the blood volume of a 400 kg rat. This Vd indicated extensive distribution to the tissues. No dose-related change in the Vd was observed. Distribution of styrene to the major organs was fairly rapid and uniform for a 13.37 mg/kg intravenous dose. This dose resulted in higher apparent organ-to-blood concentration ratios than a lower dose of 4.011 mg/kg for most organs and sampling times.

Results from inhalation studies also indicate that distribution of styrene is widespread with relatively high concentrations in adipose tissue.

Withey and Collins (1979) compared organ/blood concentration ratios in rats after 5 hours of inhalation exposure to styrene concentration ranging from 50-2000 ppm. The pattern of styrene distribution to the organs changed as did a number of the organ/blood ratios with changes in the exposure level. As the exposure level increased, the relative concentration decreased in the kidney, but increased in the liver and brain. Perirenal fat contained a relatively high concentration of styrene (at least 10 times greater than other organs), which was expected based on the high oil:blood partition coefficient estimated as 130 or 105 by Van Rees (1974) and Sato and Nakajima (1979), respectively.

In rats exposed to 80 or 1200 ppm by inhalation for ≤24 hours, the styrene concentration in fat over time was consistent with the pattern expected for the second (peripheral) compartment of a two-compartment toxicokinetic model (Ramsey and Young, 1978; Young et al., 1979). Measurements indicated that most of the peripheral compartment consists of fat. The fat:blood ratio was 40:1 and 80:1 at 80 and 1200 ppm, respectively (Ramsey and Young, 1978). The fat concentration of styrene increased 153-fold from 34-5200 μg/g with the 15-fold increase in exposure level (Young et al., 1979).

Ramsey and Andersen (1984) developed a physiologically-based pharmacokinetic model that described the behavior of inhaled styrene in rats and predicted that styrene would behave the same way in humans. The objective of the model was to simulate the concentrations of styrene in both blood and fat over an entire range of concentrations using a single set of physiological and biochemical constants. In this model quantifications are based on the time course of styrene concentrations distributed to four main tissue groups: 1) the fat tissue group representing total adipose tissue, 2) the muscle tissue group including skin, 3) richly perfused tissues such as the kidney, brain and viscera, and 4) the liver as well as other tissues with significant metabolic capacity for styrene. Using the data of Young et al. (1979), the investigators adequately simulated the styrene concentrations in blood and fat of rats exposed to concentrations of styrene between 80 and 1200 ppm. It was found that the simulated behavior of styrene is sensitive to changes in the constants describing the fat tissue, blood perfusion and flow rate, metabolism rate and fat:blood partition coefficient. The behavior of inhaled styrene in humans was predicted by scaling-up the constants used to successfully simulate styrene behavior in rats. Blood concentrations in rats, mice and humans were nearly identical at inhaled styrene concentrations of ≤200 ppm. Ramsey and Andersen (1984) concluded that based on model simulations, laboratory rodents exposed to concentrations of <200 ppm are reasonable models for estimating blood concentrations in humans and that blood concentrations are linearily related to the inhaled exposure concentrations ≤200 ppm.

Immediately following a 4-hour inhalation exposure of rats to 500-1000 ppm styrene, the largest organ/blood concentration ratio was for adipose tissue (32), followed by liver (3) (Teramoto and Horiguchi, 1979, 1981). Lower levels were found in the brain, kidney, spleen and muscle. A similar pattern was observed following intraperitoneal injection of 350 mg/kg styrene (Teramoto and Horiguchi, 1981).

Kidneys contained the largest concentration of styrene and its metabolites after inhalation exposure of rats to 43.5 ppm 7-^{14}C-styrene for 1-8 hours (Carlsson, 1981). Subcutaneous fat, liver and ischiadic nerve also contained relatively large concentrations. Radioactivity was detected in all 10 organs and tissues examined. Kidney and subcutaneous fat concentrations rose rapidly for the first 2-4 hours of exposure, then exhibited more gradual increases.

Following inhalation of 8-^{14}C or ring-^{14}C-styrene (10 μℓ of 3 μCi/μℓ) for 10 minutes by mice, low temperature, whole-body autoradiography revealed a high uptake of radioactivity by adipose and nervous tissues, and blood, liver, kidney and lung (Bergman, 1979).

Styrene was rapidly distributed to all tissues examined in mice after intraperitoneal injection of 200 mg/kg styrene in oil (Pantarotto et al., 1980). High concentrations (>200 μg/g) persisted for ~2 hours in perirenal fat. Savolainen and Vainio (1977) studied the distribution of styrene and styrene oxide in rats following intraperitoneal injection of 577 μmol of ^{14}C-styrene in oil (60 mg/mℓ) and 460 μmol ^{3}H-styrene oxide (55 mg/mℓ). The locations of the label in the molecule were not stated. The duodenum, kidney, liver and brain contained high levels of radioactivity relative to blood for both styrene and styrene oxide.

Measurable quantities of styrene oxide were reported to accumulate in kidneys, lungs, livers and blood of NMRI mice following styrene exposure (Lõf et al., 1984).

The distribution of styrene in pregnant rats was studied by Grice et al. (1981). Exposure of pregnant rats to 2000 ppm styrene for 5 hours on day 17 of gestation resulted in mean levels of ~38-65 μg/g in the offspring that confirmed transplacental transfer of styrene.

In humans, Ramsey and Young (1980) calculated an average apparent Vd for the blood of 1350±360 g/kg (units were given by the authors) for volunteers exposed for 6 hours to 80 ppm styrene.

The only tissue concentration data for humans is provided by biopsies of subcutaneous adipose tissue from volunteers following inhalation exposure. Mean concentrations of styrene in adipose tissue samples were ~3.6 mg/kg (~8% of total absorption) throughout the first 24 hours after exposure of subjects to 50 ppm styrene during four 30-minute periods of rest and exercise (Engstrom et al., 1978a). The mean daily uptake in adipose tissue of three workers exposed to TWA concentrations of 7.6-20.0 ppm was 193-558 mg (Engstrom et al., 1978b). Wolff et al. (1977) detected styrene in fat biopsy samples from 13 of 25 styrene polymerization workers.

Dowty et al. (1976) compared volatile components in maternal and cord blood in gravid women patients. Concentrations of transplacental styrene was somewhat higher (levels not reported) than those of maternal blood, which suggested a selective one-way transplacental transfer. Pellizzari et al. (1982) detected styrene in 8/8 milk samples collected from lactating women.

Retention

Results from a number of studies indicate that styrene is relatively rapidly eliminated from all tissues in animals. Only limited data are available for humans.

At 24 hours following oral administration of 20 mg/kg ^{14}C-styrene (uniform ring-labelled) to rats, concentrations in all tissues and organs tested were <1 µg/g (Plotnick and Weigel, 1979). Peak levels occurred at or before 4 hours. The organs with the highest concentrations of radioactivity at all time intervals tested (2, 4, 8, 12 and 24 hours) were the kidney>liver>pancreas (Plotnick and Weigel, 1979). No styrene was detected at 48 hours. Sauerhoff et al. (1976) detected no radioactivity in tissues 72 hours after oral dosing of rats with 50 or 500 mg/kg ^{14}C-styrene.

The elimination of styrene from the heart, brain, liver, spleen and kidney of rats was described by biphasic log-linear kinetics after intravenous injection of 4.011 mg/kg (Withey and Collins, 1977). Half-lives (calculated from the author's kinetic parameters) ranged from 3.8-7.1 minutes for the alpha (fast) phase and from 20-37 minutes for the beta (slow) phase. Linear elimination was observed during the 90-minute monitoring period for a higher dose of 13.37 mg/kg.

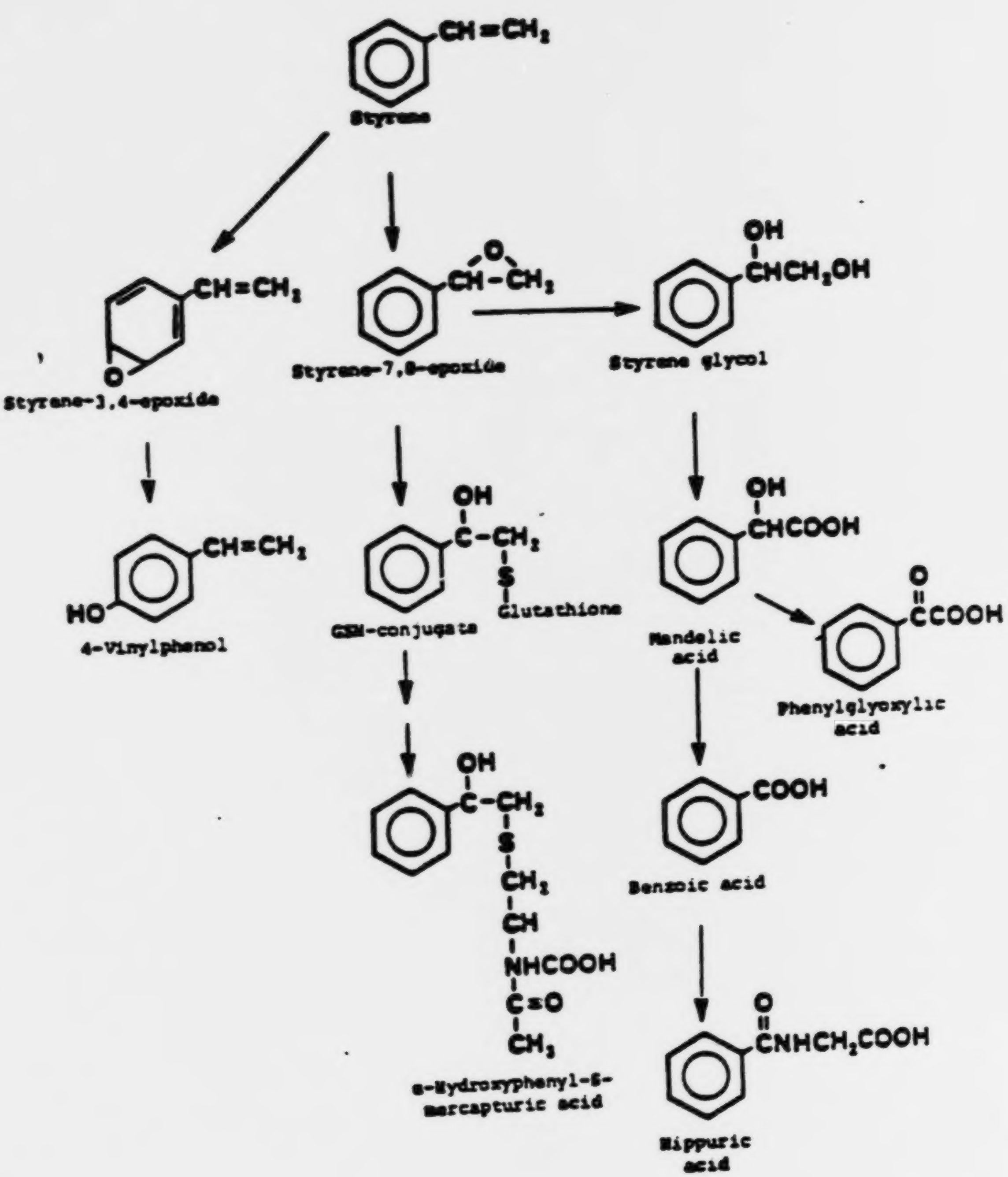
After a 4-hour inhalation exposure of rats to 500-1000 ppm styrene, Teramoto and Horiguchi (1979, 1981) determined biological half-lives ranging from 2-2.5 hours in all tissues examined except adipose tissue, which had a half-life of 6.3 hours. No significant accumulation of styrene was found after 5 daily 4-hour exposures to 700 ppm. Carlsson (1981) estimated the half-life of styrene and its metabolites to be 2.2 hours in subcutaneous fat of rats following a 1-hour inhalation exposure to 43.5 ppm 7-^{14}C-styrene. Low temperature autoradiography results indicated that styrene is cleared from nervous tissues of mice in 1 hour following inhalation exposure (10 μℓ of 3 μCi/μℓ for 10 minutes) (Bergman, 1979). At 12 hours after intraperitoneal injection of 200 mg/kg styrene, trace levels were detected only in perirenal fat of mice; nc styrene was detected in the other tissues examined (Pantarotto et al., 1980).

In humans exposed to 50 ppm styrene for 2 hours, Engstrom et al. (1978a) estimated half-lives for styrene in adipose tissue of 2-4 days. Styrene was detected in 1 subject 13 days after exposure.

Predictions based on a toxicokinetic model (parameters estimated from a human inhalation study) indicated that maximum concentrations of styrene in both blood and fat of humans are reached after a few repeated 8-hour daily exposures to 80 ppm styrene, suggesting no tendency for long-term accumulation (Ramsey and Young, 1978, 1980; Ramsey et al., 1980).

Metabolism

The metabolic fate of styrene in mammals has been studied extensively. There is limited information from human studies, but similarities to the process in other mammals have been identified. Some metabolic pathways of styrene are given in Figure III-1. This scheme is based on reviews of early studies.



CH=CH$_2$
Styrene
CH=CH$_2$
O
Styrene-3,4-epoxide
O
CH-CH$_2$
Styrene-7,8-epoxide
OH
CHCH$_2$OH
Styrene glycol
CH=CH$_2$
HO
4-Vinylphenol
OH
C-CH$_2$
S
Glutathione
GSH-conjugate
OH
CHCOOH
Mandelic acid
O
CCOOH
Phenylglyoxylic acid
OH
C-CH$_2$
S
CH$_2$
CH
NHCOOH
C=O
CH$_3$
α-Hydroxyphenyl-S-mercapturic acid
COOH
Benzoic acid
O
CNHCH$_2$COOH
Hippuric acid

Figure III-1

Metabolic Pathways of Styrene

Source: Adapted from Ohtsuji and Ikeda, 1971; Leibman, 1975; NIOSH, 1983

Intraperitoneal injection of styrene-7,8-oxide or styrene glycol (phenylethylene glycol) in rats resulted in excretion of mandelic, phenylglyoxylic and hippuric acids, and glucuronide (Ohtsuji and Ikeda, 1971). Injection of styrene resulted in the same urinary metabolites. Injection of mandelic acid yielded all three acids, while only phenylglyoxylic acid was excreted following phenylglyoxylic acid injection. Pretreatment with phenobarbital, an inducer of microsomal cytochrome P-450, stimulated metabolism of styrene but had no effect on styrene oxide or styrene glycol metabolism. The metabolism of styrene was inhibited by SKF 525A, the microsomal oxidation inhibitor (Ohtsuji and Ikeda, 1971).

Leibman and Ortiz (1969) found that styrene (50 μmol) was converted to styrene glycol at a rate of 78 nmol product/hour for rat liver 9000 x g supernatant fraction (S-9 fraction containing both microsomal and cytosolic enzymes) prepared from animals pretreated with phenobarbital. The conversion rate was 55 nmol product/hour for a similar rabbit liver fraction. Gas chromatographic results of experiments using the S-9 fraction from phenobarbital pretreated rabbits indicated that styrene-7,8-oxide was formed from styrene only when NADPH-generating cofactors were present in the incubation mixture (Leibman and Ortiz, 1970). Microsomes were capable of catalyzing the conversion of styrene to styrene oxide in the absence of cytosol. Saturation of the enzyme converting styrene to styrene glycol resulted in the accumulation of ^{14}C-styrene oxide when 8-^{14}C-styrene was incubated with the S-9 fraction. These results indicated that styrene was converted to styrene oxide by microsomal oxygenase (Leibman and Ortiz, 1970). Styrene oxide, in turn, was converted by a microsomal epoxide hydrase to styrene glycol (Leibman and Ortiz, 1968).

Styrene-7,8-oxide can also conjugate with reduced glutatione (GSH) to form hydroxyphenyl mercapturic acids observed in vivo by James and White (1967). Cytosolic glutathione-S-transferase catalyzed this conjugation in vitro (Boyland and Williams, 1965; Fjellstedt et al., 1973). The stereoselectivity of this reaction has been studied intensively (Pachecka et al., 1979; Seutter-Berlage et al., 1978; Delbressine et al., 1981; Watabe et al., 1981, 1982a, 1983).

The two metabolic pathways discussed above are considered the major pathways in styrene metabolism. Detection of additional urinary metabolites has led to the proposal of several minor pathways. Identification of mercaptoacetic acid in rats (Nakatsu et al., 1980) and guinea pigs (Horning et al., 1981) dosed with styrene oxide indicates that GSH conjugates can be metabolized to mercaptoacetic acid in addition to mercapturic acid derivatives. Styrene glycol acetate was formed by rabbit liver microsomal action on styrene oxide or styrene glycol (Battistini et al., 1979). Deuterated phenaceturic acid (a glycine conjugate of phenylacetic acid) was detected in the urine of rats administered deuterated styrene (Delbressine et al., 1980). This metabolite was proposed to arise either from intramolecular rearrangement of styrene oxide to phenylacetaldehyde or from oxidation of 2-phenylethanol to phenylacetaldehyde. The aldehyde could be oxidized to phenylacetic acid and following conjugation with glycine be excreted as phenaceturic acid.

Bakke and Scheline (1970) detected low levels of 4-vinyl phenol (<0.1% of dose) in urine following oral administration of styrene to rats. Pantarotto et al. (1978) demonstrated indirectly that styrene-3,4-oxide was an intermediate in the formation of styrene phenolic derivatives. Further support for the proposed intermediacy of styrene-3,4-oxide in styrene metabolism is given by Watabe et al. (1982b). The authors synthesized styrene-3,4-oxide, which was rapidly converted to 4-vinyl phenol in water and phosphate buffer at physiological pH (half-time of formation was 4.3 seconds). Pfaffli et al. (1981) identified 4-vinyl phenol in the urine of 29/29 plastic workers examined (TWA exposure=130 ppm styrene), and suggested styrene-3,4-oxide is formed as an intermediate product in humans. The mean 4-vinyl phenol/mandelic acid excretion ratio was ~0.3% indicating the pathway to 4-vinyl phenol is relatively minor. The 3,4-epoxide, which has been shown to be a potent mutagen and the most reactive benzene oxide ever synthesized, is probably the metabolic intermediate (Watabe et al., 1982b).

Mandelic acid and phenylglyoxylic acid have been identified as the primary urinary metabolites of styrene in humans (Bardodej and Bardodejova, 1970; Astrand et al., 1974; Guillemin and Bauer, 1978, 1979). Guillemin and Bauer (1979) exposed human volunteers to styrene at levels of 50, 100 or 200 ppm and found that of the 89.9% absorbed dose excreted in the urine, 33% was phenylglyoxylic acid and 56.9% was mandelic acid. Some hippuric acid has been detected, but levels are quite variable and are usually within basal limits (Leibman, 1975; Guillemin and Bauer, 1979).

The major site of styrene metabolism is the liver, but styrene mono-oxygenase, glutathione-S-transferase and epoxide hydrase activities have been detected in other organs (Pacifici et al., 1981; Cantoni et al., 1978; Ryan et al., 1976; Salmona et al., 1976). The oxidation of styrene to styrene oxide in vitro has been catalyzed by heme proteins other than cytochrome P-450 (Belvedere et al., 1982; Cantoni et al., 1982; Belvedere and Tursi, 1983). Epoxide hydrase and mono-oxygenase activities have been associated with the nuclear membranes of in vitro rat hepatocytes (Bornstein et al., 1978; Garattini et al., 1981; Gazzotti et al., 1980, 1981).

Belvedere and Tursi (1981) found that human erythrocytes and lymphocytes were capable of metabolizing styrene to styrene oxide in vitro. The erythrocyte catalyzed oxidation was not affected by the addition of NADPH and NADH, was inhibited by carbon monoxide and was undetectable when oxygen was not present. These results suggested to the authors that oxyhemoglobin may be involved in the reaction.

With styrene oxide as a substrate, Pacifici and Rane (1982) measured significant rates of epoxide hydrolase and glutathione-S-transferase activity in microsomes isolated from aborted human fetal tissues at 14-25 weeks of gestation. Epoxide hydrolase activity was highest in the liver and adrenals. Comparable glutathione-S-transferase activity was observed in 5/6 of the tissues examined, with lower activity in the intestine.

In summary, both major metabolic pathways involve the metabolism of styrene by the microsomal NADPH-cytochrome P-450 dependent mono-oxygenase to styrene oxide, a highly reactive metabolite believed to be an ultimate toxicant. Styrene oxide can then be hydrated to styrene glycol. This reaction is catalyzed by microsomal epoxide hydrase. Styrene glycol, in turn, can be metabolized to mandelic, phenylglyoxylic, benzoic or hippuric acids, or excreted as a glucuronide conjugate. Detoxification of styrene oxide can also occur by the action of cytosolic glutathione-S-transferase in the presence of GSH resulting in mercapturic acids as urinary metabolites (Leibman, 1975; Delbressine et al., 1981).

Elimination

The elimination of styrene from various tissues has been described in the Retention Section.

Sauerhoff et al. (1976) found dose and sex-dependent differences in the elimination of uniform ring-^{14}C-styrene administered to rats in a single oral dose. Over a 72-hour collection period, 95% of a 50 mg/kg dose was excreted in the urine, 1% was expired and 4% was recovered in the feces. For a 500 mg/kg dose, 90% was located in the urine, 9% was expired and 1.5% was excreted in the feces. Less than 0.1% of the radioactivity was expired as $^{14}CO_2$. Biphasic urinary excretion was observed for the 50 mg/kg dose with half-lives of 1.29±0.19 and 8.13±1.22 hours for the alpha and beta phases, respectively. For the 500 mg/kg dose, excretion of styrene reached a peak between 12 and 24 hours and then declined linearly with a half-life of 6.74±0.67 hours. The shape of this elimination curve suggested saturation of excretion or metabolism or delayed absorption. Mandelic, phenylglyoxylic, hippuric and benzoic acids were identified as 4 of the 7 urinary metabolites of styrene detected. Dose-related alterations in the relative percentages of hippuric and mandelic acid were also suggestive of saturated metabolism. Clearance of radioactivity from plasma was monophasic during the first 32 hours after dosing, resulting in half-lives of 5.13±0.16 and 14.3±1.6 hours for the 50 and 500 mg/kg doses, respectively.

Withey (1976) obtained a biphasic blood elimination curve with half-lives for the alpha and beta phase of 16.9 and 47.2 minutes, respectively.

after intragastric administration of a 3.147 mg dose in aqueous solution. Withey (1976) followed styrene to lower concentration in the blood than Sauerhoff et al. (1976), which may explain the difference in results (biphasic vs. monophasic curves).

In 24 hours, 90% of a 20 mg/kg oral dose of uniform ring-label-^{14}C-styrene given to rats was excreted in the urine; <2% was excreted in the feces (Plotnick and Wiegel, 1979).

The elimination data Sauerhoff and Braun (1976) obtained following inhalation exposure of rats to 60 or 600 ppm uniform ring-^{14}C-styrene for 6 hours were similar to the data from their oral study (Sauerhoff et al., 1976). Approximately 90% of the radioactivity was recovered as nonvolatile urinary metabolites, 6% was expired and 2.3% was recovered in feces. Relative percentages of three metabolites changed with exposure level. Biphasic urinary excretion was evident at both exposures.

After 10-minute exposure of mice to 8-^{14}C-styrene (10 µℓ of 0.169 µCi/µℓ), ~7% of the calculated dose was eliminated as $^{14}CO_2$, while 2.9% was eliminated in the air as unchanged styrene. Elimination in exhaled air was biphasic with apparent half-lives for the alpha and beta phases of 15.5 and 275.8 minutes, respectively (Bergman, 1979).

Ramsey and Young (1978, 1980) and Young et al. (1979) found that the blood elimination curve for rats was biphasic log-linear at low exposure concentrations (80 and 220 ppm styrene for 6 hours), which was consistent with a two-compartment toxicokinetics model. At higher exposures (≥600 ppm for 6 hours), a nonlinear blood elimination curve indicative of Michaelis-Menton kinetics for saturated systems was obtained. The area under the blood concentration curve (AUC) from 0-24 hours reflected the magnitude of the saturation effect on the concentration-time relationship. A 15-fold increase in exposure level (80-1200 ppm) resulted in a 112-fold AUC increase.

Teramoto and Horiguchi (1979, 1981) also found that the clearance of styrene from blood exhibited saturation kinetics in rats exposed to 700 ppm styrene for 4 hours. Withey and Collins (1977, 1979) fit biphasic log-linear blood eliminaton curves to the data at all concentrations and doses tested in rats ($\leq$2000 ppm for 5-hour inhalation exposure and 9.36 mg/kg intravenous dose). The estimated values of the rate coefficients for the two compartment toxicokinetic model tended to decrease as the dose or exposure increased, but the apparent volume of distribution calculated in the intravenous study did not change with dose. Withey and Collins (1977) suggested this behavior could be explained by the involvement of saturable metabolic pathways.

In humans, Astrand et al. (1974) observed biphasic elimination of mandelic acid that continued for 9-13 hours following inhalation exposure of volunteers to 50 or 150 ppm styrene for 30 minutes. Mandelic acid accounted for 50% of the absorbed dose, which contrasts with the findings of Bardodej and Bardodejova (1970) that 85% of the retained dose of inspired styrene in humans was excreted as mandelic acid and 10% as phenylglyoxylic acid. Astrand et al. (1974) suggested that the percentage given by Bardodej and Bardodejova (1970) may not be very accurate because of the failure to correct for basal mandelic acid levels and a selection of a low concentration of styrene (22 ppm).

Guillemin and Bauer (1978, 1979) exposed human subjects to concentrations of 50, 100 or 200 ppm styrene for 4-8 hours. The metabolites mandelic and phenylglyoxylic acid were present in urine within 1 hour after exposure began. Maximal excretion occurred at termination of exposure for mandelic acid and 2 hours following termination for phenylglyoxylic acid. Excretion of mandelic acid was biphasic with average half-lives of 3.9±0.9 and 24.7±10.3 hours for the alpha and beta phases, respectively. Excretion of phenylglyoxylic acid was linear for 50 hours after exposure with a half-life of 10.5 hours. The excretion rates were independent of exposure level and duration. The authors believed phenylglyoxylic acid elimination to be biphasic (with the second phase beginning after 50 hours) since mandelic acid is a precursor of phenylglyoxylic acid. Mandelic and phenylglyoxylic acid excretion accounted for 57 and 33%, respectively, of the retained styrene dose.

A two-compartment linear toxicokinetic model was used to describe the disposition of styrene in humans exposed to inhalation of 80 ppm styrene for 6 hours (Ramsey and Young, 1978, 1980; Ramsey et al., 1980). Clearance of styrene from the blood was biphasic with average half-lives of 0.58±0.08 and 13.0±0.8 hours for the alpha and beta phases, respectively. Elimination of styrene from the lungs also followed biphasic log-linear kinetics. Urinary metabolites (mandelic and phenylglyoxylic acid) accounted for ~97% of styrene clearance, while the remaining 2-3% was expired.

Elimination of styrene in the lungs was biphasic and rapid, with half-lives of 40 and 180 minutes for the alpha and beta phases, respectively, in an inhalation study of three workers exposed to 80 ppm styrene for 5 hours

(Teramoto and Horiguchi, 1979). Engstrom et al. (1978a) exposed subjects to 210 mg/m^3 of styrene in air for four 30-minute periods of rest or exercise. During the first 4 hours after exposure, ~2% of the retained styrene was expired. By 19 hours postexposure, 2.6% had been expired and the concentration in alveolar air was near the limit of detection.

Following dermal exposure of humans to styrene [liquid styrene and aqueous solutions of 66.5-269 mg/ℓ (66.5-269 ppm) applied to forearms], only ~13% of the absorbed dose was excreted as mandelic acid (Dutkiewicz and Tyras, 1968). This value is considerably less than reported by Astrand et al. (1974) for inhalation. In another dermal study (skin exposure to vapor concentrations of 600 ppm for 3.5 hours), Riihimaki and Pfaffli (1978) calculated that 90.5% of the absorbed dose was excreted in the urine and the remaining 9.5% expired.

The kinetics of styrene elimination appear to be similar for humans and animals at low doses. The elimination kinetics in rats at high doses or exposures (between 50 and 500 mg/kg oral; between 200 and 600 ppm inhalation) are indicative of saturated metabolism. These results indicated to Young et al. (1979) that data from rats exposed to low doses (exhibiting linear pharmacokinetics) should be extrapolatable to humans, but such extrapolations from high dose studies (exhibiting saturable metabolism or elimination) may not be appropriate.

Summary

Styrene was readily absorbed from the GI tract of rats. Peak plasma levels were reached in rats within minutes following a dose in aqueous solution and in <2 hours after a dose in oil (Withey, 1976). Almost complete absorption of oral doses of styrene is indicated by the recovery of 90-95% of the dose as urinary metabolites (Plotnick and Wiegel, 1979; Sauerhoff et al., 1976). Human data are lacking on absorption following oral dosing. Significant dermal absorption has been demonstrated in humans and rats (Dutkiewicz and Tyras, 1968; Tsurata, 1982; Shugaev, 1969). Absorption of styrene in the lungs is rapid in both rats and humans (Withey, 1976; Withey and Collins, 1979; Ramsey and Young, 1978, 1980; Astrand et al., 1974). Direct human evidence indicates that styrene-7,8-oxide can be measured (although at the detection limit in most cases) in the blood of workers exposed to styrene in glass-fiber reinforced plastics factories (Löf et al., 1986).

Animal studies indicate that distribution of absorbed styrene is widespread and rapid. Peak tissue concentrations were reached as early as 2-4 hours after oral exposure (Plotnick and Wiegel, 1979). Organ/blood concentration ratios changed with dose or exposure level (Withey and Collins, 1977, 1979; Ramsey and Young, 1978; Young et al., 1979). Relative concentrations of styrene increased in the liver and brain with increased exposure (Withey and Collins, 1979). Relatively high concentrations of styrene have been found in adipose tissue (Teramoto and Horiguchi, 1979, 1981; Withey and Collins, 1979; Carlsson, 1981; Bergman, 1979). Fat has been proposed as the peripheral compartment of a two-compartment toxicokinetic model for styrene (Ramsey and Young, 1978; Young et al., 1979).

Styrene is fairly rapidly eliminated from animal tissues. Concentrations in all tissues tested were <1 μg/g 24 hours after oral dosing (Plotnick and Wiegel, 1979). Elimination from fat lags behind other tissues (Pantarotto et al., 1980; Teramoto and Horiguchi, 1979, 1981). Repeated exposure did not result in styrene accumulation (Teramoto and Horiguchi, 1979, 1981). In humans, Engstrom et al. (1978a) estimated half-lives in adipose tissue of 2-4 days.

The two major metabolic pathways both involve the conversion of styrene to styrene oxide by microsomal oxygenase (Leibman and Ortiz, 1970). Styrene oxide is a highly reactive intermediate product believed to be an ultimate toxicant. Microsomal epoxide hydrase catalyzes the conversion of styrene oxide to styrene glycol, which is further metabolized to the urinary metabolites glucuronide or mandelic, phenylglyoxylic, benzoic or hippuric acid (Ohtsuji and Ikeda, 1971; Leibman, 1975). An alternate pathway involves the conjugation of styrene oxide with reduced glutathione (GSH) to form mercapturic acids (Boyland and Williams, 1965; Fjellstedt et al., 1973). Several minor pathways have also been proposed (Delbressine et al., 1980; Pantarotto et al., 1978; Watabe et al., 1982b).

Styrene is excreted almost completely (90-97%) as urinary metabolites in both rats (Plotnick and Wiegel, 1979; Sauerhoff et al., 1976) and humans (Guillemin and Bauer, 1978, 1979; Ramsey and Young, 1978, 1980; Ramsey et al., 1980). In animals, elimination of styrene in expired air, urine and from the blood is rapid and biphasic log-linear at low doses (Sauerhoff et al., 1976; Withey, 1976; Sauerhoff and Braun, 1976; Bergman, 1979; Ramsey and Young, 1978, 1980; Young et al., 1979; Withey and Collins, 1977, 1979). At higher doses, the elimination curves indicate saturation of metabolism or excretion (Sauerhoff et al., 1976; Ramsey and Young, 1978, 1980; Young et al., 1979; Teramoto and Horiguchi, 1979, 1981). Biphasic elimination is also observed in humans with plasma half-lives of 0.6 and 13 hours for the alpha and beta phases, respectively (Ramsey and Young, 1978, 1980; Ramsey et al., 1980) and urinary mandelic acid alpha and beta half-lives of ~4 and 25 hours, respectively (Guillemin and Bauer, 1978, 1979).

IV. HUMAN EXPOSURE

This chapter will be submitted by the Science and Technology Branch, Criteria and Standards Division, Office of Drinking Water.

V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Acute Toxicity. Acute toxic levels of styrene by different routes of administration are summarized in Table V-1.

Wolf et al. (1956) reported an acute oral LD_{50} of ~5000 mg/kg for rats treated by gavage, which indicates that the acute toxicity of styrene is relatively low. The lowest single oral dose of styrene (administered by oral intubation) causing 100% mortality in rats within 2 weeks of treatment was 8000 mg/kg, while 1600 mg/kg was the maximum dose resulting in no deaths (Spencer et al., 1942).

Harton and Rawl (1976) applied styrene dermally to the trunks of New Zealand albino rabbits at 1000, 3300, 10,000 and 20,000 mg/kg for 24 hours. No mortality occurred at any dose level within 14 days of treatment. Skin irritation did occur, and the two highest doses resulted in narcosis lasting 24-48 hours. Temporary paralysis of the posterior limbs was observed in one rabbit dosed with 20,000 mg/kg. Marked irritation with some denaturation was observed following two applications of styrene (volume not stated) bandaged onto the shaved abdomens of rabbits (Spencer et al., 1942).

Results of inhalation studies also indicate that the acute toxicity of styrene is relatively low (Table V-1). LC_{50} values for rats exposed to styrene have been reported as 11.5 mg/ℓ (2700 ppm) for 4 hours (Jaeger et al., 1974), 11.8 mg/ℓ (2770 ppm) for 4 hours (Shugaev, 1969) and 19.65 mg/ℓ (4618 ppm) for 6 hours (Bonnet et al., 1982). For mice, reported LC_{50} values are 21.0 mg/ℓ (4930 ppm) for 4 hours (Shugaev, 1969) and

TABLE V-1

Acute Toxicity of Styrene

Route	Species	Sex	Number	Dose	Effect	Reference
oral	rat	male/ female	57	5000 mg/kg	approximate LD_{50}	Wolf et al., 1956
oral	rat	NR	59	1600 mg/kg	100% survival	Spencer et al., 1942
oral	rat	NR	59	8000 mg/kg	100% mortality	Spencer et al., 1942
oral	rat	NR	NR	8000 mg/kg	lethal concentration	Faustov, 1960
inhalation	rat	male	12	19.65 mg/ℓ (4618 ppm) x 6 hours	LC_{50}; death usually occurred within 24 hours	Bonnet et al., 1982
inhalation	rat	NR	405	6.3 mg/ℓ (1400 ppm)	100% survival for 3-hour exposure; 100% mortality for >40-hour exposure	Spencer et al., 1942
inhalation	rat	NR	405	46.4 mg/ℓ (11,000 ppm)	100% survival for 1 hour; 100% mortality for 3 hours	Spencer et al., 1942
inhalation	rat	NR	NR	11.5 mg/ℓ (2700 ppm) x 4 hours	LC_{50}; death was due to pulmonary irritation and edema	Jaeger et al., 1974
inhalation	rat	NR	NR	11.8 mg/ℓ (2770 ppm) x 4 hours	LC_{50}	Shugaev, 1969
inhalation	guinea pig	NR	410	6.3 mg/ℓ (3100 ppm)	100% survival for 16-hour exposure; 100% mortality for 40-hour exposure	Spencer et al., 1942
inhalation	guinea pigs	NR	410	46.4 mg/ℓ (10,000 ppm)	100% survival for 1 hour; 100% mortality for 3 hours	Spencer et al., 1942
inhalation	mouse	NR	NR	21.0 mg/ℓ (4930 ppm) x 4 hours	LC_{50}	Shugaev, 1969

TABLE V-1 (cont.)

Route	Species	Sex	Number	Dose	Effect	Reference
inhalation	mouse	NR	NR	10.34 mg/ℓ (2429 ppm) x 6 hours	LC_{50}; death usually occurred within 24 hours	Bonnet et al., 1982
inhalation	mouse	NR	NR	15.0 mg/ℓ (3522 ppm) x 2 hours	30% mortality	Blinova and Rylova, 1957
inhalation	mouse	NR	NR	10.0 mg/ℓ (2348 ppm) x 2 hours	20% mortality	Blinova and Rylova, 1957
inhalation	mouse	NR	NR	2.5 mg/ℓ (586 ppm) x 5 minutes	RD_{50}*	De Ceaurriz et al., 1981
inhalation	mouse	NR	NR	0.67 mg/ℓ x 3 minutes (157 ppm)	RD_{50}*	Alarie, 1973
inhalation	mouse	NR	NR	4.2 mg/ℓ (980 ppm)	RD_{50}*	Alarie, 1981

*RD_{50} = a 50% decrease in the respiratory rate

NR = Not reported

10.34 mg/ℓ (2429 ppm) for 6 hours (Bonnet et al., 1982). Based on these data, no marked differences in susceptibilities to styrene between rats and mice are apparent.

The acute toxicity of styrene oxide, a highly reactive styrene metabolite, is greater than for styrene. An oral LD_{50} of styrene oxide in rats has been reported as 2550 mg/kg (Weil et al., 1963). An approximate dermal LD_{50} value for styrene oxide of 800 mg/kg was calculated by the researchers after application of styrene oxide to the shaved skin of New Zealand rabbits for 24 hours. They also noted that 2/6 rats died after exposure to styrene oxide at 4914 mg/m³ (1000 ppm) for 4 hours. In contrast, all rats exposed to styrene at ~6000 mg/m³ (1400 ppm) for 30 hours survived (Spencer et al., 1942).

Ohtsuji and Ikeda (1971) compared the toxicities of styrene and styrene oxide in rats following intraperitoneal injection. No sex-related differences in susceptibility were evident. The LD_{50} values for styrene determined within 48 hours of injection ranged from 2150-2860 mg/kg. The LD_{50} values for styrene oxide were 4-5 times lower, ranging from 460-610 mg/kg.

Two drops of undiluted styrene applied directly to the eyes of rabbits resulted in moderate conjunctival irritation, including inflammation and swelling of the eyelid, but corneal injury was very slight (Wolf et al., 1956).

Spencer et al. (1942) reported that the initial response to inhalation of styrene in rats and guinea pigs was eye and nasal irritation, with excess salivation, lachrymation and nasal discharge. A dose-related loss of equilibrium progressed to unconsciousness at high exposures. In both rats and guinea pigs, unconsciousness occurred within 24-30 hours, 10-12 hours, 1 hour and 1 hour for exposures of 9.3, 11.6, 23.2 and 46.4 mg/ℓ (2200, 2700, 5450 and 11,000 ppm), respectively. Exposure-related histological damage in rats was concentrated in the lungs, but some liver and kidney changes were reported. Styrene levels as low as 6.0 mg/ℓ (1400 ppm) produced lesions in the lung.

Subchronic and Chronic Toxicity.

Oral Exposure. Oral studies of subchronic and chronic toxicity of styrene are summarized in Table V-2. In an early study, male rats were administered doses of 100, 500, 1000 and 2000 mg/kg of styrene 5 days/week for 4 weeks (Spencer et al., 1942). There were no apparent toxic effects and no significant pathological lesions at autopsy at the lowest dose. At 500 mg/kg, weight gains were reduced, but no significant hematologic or pathologic effects were evident. A few deaths (unspecified number), attributed to severe irritation of the esophagus and stomach, occurred at the two highest doses.

Wolf et al. (1956) administered doses of 66.7, 133, 400 and 667 mg styrene/kg to rats by stomach tube 5 times/week for 6 months. No histopathologic or hematologic effects were noted at any dose level, but the two highest doses resulted in depressed growth and increased kidney and liver weight.

TABLE V-2

Subchronic and Chronic Toxicity of Orally Administered Styrene

Species/ Strain	Sex/No.	Age or Weight	Vehicle	Dose	Duration	Effect	Reference
Rats/NR	male 5/group*	NR	NR	100, 500, 1000, 2000 mg/kg	5 days/week 4 weeks	100 mg/kg: no apparent toxic effects, no pathological lesions; 500 mg/kg: reduced weight gains, no significant hematologic or pathologic effects; 1000 and 2000 mg/kg: a few deaths (unspecified number) attributed to pronounced irritation of the esophagus and stomach	✓Spencer et al., 1942
Rat/Wistar-derived	female 10/group 20 control	~2 months	olive oil/ gum arabic	0, 66.7, 133, 400, 667 mg/kg	5 days/week 6 months	66.7 and 133 mg/kg: no treatment related effects observed; 400 and 667 mg/kg: growth depression and increased liver and kidney weight, no histopathologic or hematologic effects	✓Wolf et al., 1956
Rat/NR	male NR	110 ± 5 g	ground nut oil	0, 200, 400 mg/kg/day	6 days/week 100 days	No signs of overt toxicity, no effect on body weight gain or liver weight. In the liver, significant dose-related increases in the activities of benzo[a]pyrene hydroxylase and aminopyrene-N-demethylase; dose dependent decreases in glutathione-S-transferas activity. 200 and 400 mg/kg: significant decreases ($p<0.01$) in mitochondrial succinic dehydrogenase and beta-glucuronidase activities in the liver. 400 mg/kg only: hepatic acid phosphatase activity decreased significantly ($p<0.01$), activity of SGOT and SGPT increased significantly ($p<0.02$), tiny areas of focal necrosis consisting of a few degenerated hepatocytes and inflammatory cells found in the liver (prevalence not reported). No change in hepatic glucose-6-phospatase.	✓Srivastava et al., 1982

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Age or Weight	Vehicle	Dose	Duration	Effect	Reference
Rats/NR	male 6/group	adult	nut oil	0, 270, 450, 900 mg/kg	7 days	270 mg/kg: No significant change in hepatic enzyme activities. 450 and 900 mg/kg: stimulation of aminopyrine-N-demethylase, ethylmorphine-N-demethylase, aryl hydrocarbon hydroxylase and aniline hydroxylase activities, reduced glutathione-S-transferase activity; 900 mg/kg only: significantly ($p<0.001$) lower glutathione content in the liver	Das et al., 1981a
Rats/Wistar	male 4/group	100 ± 10 g	peanut oil	0, 270, 450, 900 mg/kg	7 days	Significant($p<0.02$) decrease in brain glutathione-S-transferase activity at 450 and 900 mg/kg.	Das et al., 1981b
Rats/NR	NR 15/group	110 ± 5 g	ground nut oil	0, 450, 900 mg/kg	7 days	Significant ($p<0.01$) dose-dependent increase in renal enzyme activity (aryl hydrocarbon hydroxylase, aniline hydroxylase, benzphetamine-N-demethylase and ethylmorphine-N-demethylase). Significant ($p<0.01$) decrease in renal glutathione-S-transferase activity. Significant decrease in renal glutathione content only at 900 mg/kg.	Das et al., 1983
Rats/NR	male 5/group	200 ± 15 g	peanut oil	0,270, 450 900 mg/kg	7 days	Significant ($p<0.001$) decrease in brain aryl hydrocarbon hydroxylase at all 3 doses. Significantly ($p<0.01$) decreased brain glutathione levels at 450 and 900 mg/kg.	Dixit et al., 1982
Rats/NR	male 12/group	225-250 g	liquid styrene	0, 1 mℓ/kg (~910 mg/kg)	15 days	Dopamine levels in the brain were not affected, but noradrenaline and serotonin levels increased significantly ($p<0.001$). Brain monoamine oxidase activity decreased significantly ($p<0.001$), while acetylcholinesterase did not change.	Husain et al., 1980

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Age or Weight	Vehicle	Dose	Duration	Effect	Reference
Rat/NR	male NR; 6/group sacrificed for assay	8 weeks	peanut oil	0, 200, 400 mg/kg	90 days	No significant effect on body weight or striatal weight at either dose. Both doses resulted in a significant ($p<0.01$) increase in the specific binding of ^{3}H-spiroperidol to dopamine receptors in the corpus striatum	✓Agrawal et al., 1982
Rats/BD IV	21 pregnant rats plus male/female (144) offspring		olive oil	Single dose of 1350 mg/kg to dams on day 17 of gestation. Weekly doses of 500 mg/kg to offspring after weaning.	1/week 120 weeks	No difference in body weight. Several animals that died at 50-60 weeks had small necrotic foci in liver parenchyma and moderate lung and kidney congestion. No liver damage observed in animals that died at 80+ weeks. Lesions of kidney (hyperplasia of pelvis epithelium) and forestomach were common.	✓Ponomarkov and Tomatis, 1978
Mice/O$_{20}$	male/female 84	weaned	olive oil	1350 mg/kg	1/week 16 weeks	High early mortality among treated mice (50 and 20% mortality of males and females, respectively, at 20 weeks compared with ~100% survival for controls). Most frequent lesions in mice dying by 20 weeks were multiple centrilobular necrosis of the liver, hypoplasia of the spleen and severe lung congestion. In animals that died later, lesions were most common in the liver.	✓Ponomarkov and Tomatis, 1978
Mice/CD-1	male NR: 4/group sacrificed for each sample	7 weeks	peanut oil	0, 1, 5, 25, 50 mg/kg/day	5 days/week 1, 2 or 4 weeks	Lymphocyte formation increased significantly on splenic cell cultures after 25 mg/kg/day doses for 1 week, but a significant decrease in mitosis was observed at 50 mg/kg. After 2 weeks, cultures showed stimulated lymphocyte formation for all doses. Stimulation was less pronounced at 4 weeks; stimulation was not significant for 1 mg/kg at 4 weeks.	✓Sharma et al., 1981

TABLE V-2 (cont.)

Species/ Strain	Sex/No.	Age or Weight	Vehicle	Dose	Duration	Effect	Reference
Dogs/beagle	male/16 female/16	10 months	peanut oil	0, 200, 400, 600 mg/kg	561 days	Treatment-related alterations in body or organ weight, urinalyses or clinical chemistry determinations (serum urea nitrogen, SGPT, SGOT and AP) were not detected. Apparently, no significant effects observed at 200 mg/kg. 400 and 600 mg/kg: Red blood cells contained increased numbers of Heinz bodies, especially at 600 mg/kg and decreased red blood cell count. Minimal histopathologic changes in the liver (increased numbers of intranuclear acidophilic crystalline inclusions in the hepatocytes of the 600 mg/kg group and increased iron deposits within the reticuloendothelial cells).	Quast et al., 1978, 1979
Rats/ Sprague-Dawley	treated male/50 female/70 control male/76 female/106	35 days	drinking water	0, 7.7 and 14 mg/kg males; 0, 12 and 21 mg/kg females	2 years	No treatment-related changes in urinalysis, mortality, clinical chemistry or histology. Statistically significant reductions in water consumption for the styrene group. Relative brain weight of the high-dose female group was significantly higher than the mean of the control gorup. The mean body weight of the high-dose female group was significantly lower only during the last week of the study.	Bellies et al., 1985

*Number not specifically stated; implied to be 5

NR = Not reported

Ponomarkov and Tomatis (1978) terminated treatment after 16 weeks in a chronic exposure study with mice (weekly doses of 1350 mg/kg styrene) because of high mortality. The liver was the most common site of lesions (centrilobular necrosis in mice dying by 20 weeks); other frequently observed effects at 20 weeks were hypoplasia of the spleen and severe lung congestion.

Belles et al. (1985) administered concentrations of 0, 125 and 250 ppm styrene in drinking water to Sprague-Dawley rats (50 male, 70 female treated and 76 male, 104 female controls) for 2 years. Using water and food consumption data, corresponding doses were 7.7 and 14 mg/kg/day for males and 12 and 21 mg/kg/day for females. Longevity, physical appearance and mortality were not altered by styrene ingestion. Ocular opacities observed in all groups was judged to be not treatment related. Terminal organ weights showed only the relative brain weight of the high-dose female rats was significantly higher than the control mean. Comparison of body weight means of each treatment group with the mean of the control group revealed the body weight mean of the high-dose female group was significantly lower only at the last week of the study. Reduction in water consumption by the styrene-treated animals followed a statistically significant inverse dose-response effect and was judged to be the result of poor palatability. However, the authors determined that this reduction did not result in meaningful dehydration. Hemograms, blood chemistry and urinalysis revealed no treatment-related changes. There were also no styrene-related effects in histology or necropsy findings.

Quast et al. (1979) reported minimal toxicologic changes when beagle dogs were administered styrene for ≤561 days. These data have also been summarized in an abstract (Quast et al., 1978). Sixteen male and 16 female purebred beagle dogs were administered 0, 200, 400 or 600 mg/kg styrene by gavage as a 50/50 (w/w) mixture in peanut oil. The dogs administered 200 and 400 mg/kg styrene received doses consecutively for 561 days. The dogs in the 600 mg/kg group received their doses for 317 days, then received only peanut oil for 152 days and were returned to their doses of 600 mg/kg/day for the remaining 90 days of the study. No dose-related alterations in body or organ weights, urinalysis or clinical chemistry determinations were detected. No effects were noted at 200 mg/kg/day except for sporadic low level occurrences of Heinz bodies in the red blood cells of female dogs that were not considered treatment related. Effects in the 400 and 600 mg/kg dose groups were increased numbers of Heinz bodies in the red blood cells, decreased packed cell volume and sporadic decreases in hemoglobin and red blood counts. There were also increased iron deposits and elevated numbers of Heinz bodies found in the livers at the higher dose groups. At the 600 mg/kg/day dose, increased hepatocellular intranuclear acidophilic crystalline inclusions were detected. The authors speculated that the hepatocellular inclusions were associated with an increased rate of red blood cell removal and degradation as a result of the Heinz body. The treatment-related hematologic changes were considered to be secondarily affected by treatment and were found to be readily reversible upon discontinuing the administration of styrene. The authors concluded that the most sensitive treatment-related effects that occurred at a dose-related frequency was the formation of the intraerythrocytic Heinz body.

Das et al. (1981a) found significantly ($p<0.05$) increased activities of several mixed-function oxidase (MFO) enzymes and significantly ($p<0.05$) decreased glutathione-S-transferase activity in rats administered 450 or 900 mg styrene/kg orally for 7 days. Glutathione content was decreased at 900 mg/kg. None of the above effects were observed at 270 mg/kg.

Hepatic effects are also discussed by Srivastava et al. (1982). In this study, rats were administered styrene by gavage 6 days/week for 100 days at dose levels of 0 (vehicle control), 200 or 400 mg/kg/day. Results of mitochondrial and microsomal enzyme assays of liver tissue indicated significant dose-related alterations in the activities of a number of drug-metabolizing enzymes, including increased activity of several mixed-function oxidases and decreased activity of glutathione-S-transferase. At 400 mg/kg, focal necrosis was found. The authors noted significantly ($p<0.02$) increased activity of the serum enzymes SGOT and SGPT at 400 mg/kg, consistent with the histological evidence of hepatic focal necrosis.

Enzyme activities in the kidneys were affected similarly to hepatic enzymes. Several renal enzymes showed increased activity in rats treated orally with 450 and 900 mg styrene/kg for 7 consecutive days (Das et al., 1983). As with the liver, glutathione-S-transferase activity was significantly decreased in the kidney at both dose levels, while renal glutathione content was significantly decreased only at the high dose.

Styrene administered orally to rats at 270, 450 and 900 mg/kg for 7 days resulted in a dose-dependent decrease in brain glutathione-S-transferase activity (Das et al., 1981b). Dixit et al. (1982) also noted significantly decreased brain glutathione levels in rats treated orally for 7 consecutive days with 450 and 900 mg styrene/kg. Brain aryl hydrocarbon hydroxylase activity was significantly ($p<0.001$) decreased in rats treated with 270, 450, or 900 mg/kg (Dixit et al. 1982).

The above results indicate that styrene exposure results in increases in mixed function oxidase activity and a decrease in glutathione-S-transferase activity and glutathione content in the liver, kidney and brain. The increase in mixed function oxidase activity suggested enhanced conversion of styrene to styrene oxide (Das et al., 1981a). The decrease in glutathione-S-transferase activity suggested suppressed detoxification of styrene oxide (Das et al., 1981a,b; Srivastava et al., 1982).

Significantly elevated levels of brain noradrenaline and serotonin were observed after oral administration of ~910 mg styrene/kg to rats for 15 days (Husain et al., 1980). Dopamine levels were not altered. Brain monoamine oxidase activity decreased significantly, but there was no change in acetylcholinesterase.

Agrawal et al. (1982) administered styrene by gavage to rats at doses of 0 (vehicle control), 200 and 400 mg/kg for 90 days. Corpus striatum weights in styrene-treated rats were not significantly different from controls. Both dose levels resulted in a significant increase in the binding of ^{3}H-spiroperidol to dopamine receptors in the corpus striatum. The authors stated that the increased sensitivity of dopamine receptors suggested that the neurotoxic effects of styrene involved the dopaminergic system.

Lymphocyte formation was studied in splenic cell cultures prepared from mice treated orally with 1, 5, 25 and 50 mg styrene/kg/day for 1, 2 or 4 weeks (Sharma et al., 1981). After 2 weeks, cultures from all dose groups showed stimulated lymphocyte formation. At 4 weeks, stimulation was less pronounced. Styrene applied to splenic cultures _in vitro_ had no effect, but lymphocyte formation was stimulated by styrene oxide at concentrations as low as 2.5×10^{-7} molar.

Ponomarkov and Tomatis (1978) conducted a chronic study of styrene exposure. Twenty-one pregnant female rats were given a single dose of 1350 mg/kg. After weaning, the progeny received weekly doses of 500 mg/kg for life; surviving rats were killed at 120 weeks. Small necrotic foci in liver parenchyma and moderate lung and kidney congestion were evident in several animals that died at 50-60 weeks. Lesions of the forestomach and kidney were common (actual incidences were not reported).

Inhalation Exposure. Dow Chemical Company conducted subchronic inhalation studies of styrene summarized in Table V-3 on rats (Wistar), guinea pigs (albino, heterogeneous stock), rabbits (albino, heterogeneous stock) and rhesus monkeys (Spencer et al., 1942; Wolf et al., 1956). Rats, guinea pigs and rabbits were exposed 7-8 hours/day, 5 days/week for ~6 months. Pronounced eye and nose irritation and weight gain depression was noted in rats and guinea pigs exposed to 2000 ppm styrene, but these effects were not observed in rabbits. In rats exposed to 1300 ppm, no significant exposure-related effects on body or organ weights, gross microscopic pathology or hematology were observed, but similar irritation occurred. Approximately 10% mortality occurred in guinea pigs after the first few exposures

TABLE V-3

Summary of Dow Chemical Company Subchronic Inhalation Study[a]

Species	No./Sex[b]	Approximate Average Vapor Concentration (ppm)	Number of 7-8 hour Exposures[c]	Duration (days)	Effects[d]
Rat	28/both	2000	<105	148	Marked eye/nose irritation and weight gain depression. Listlessness and dirty/unkempt appearance. No apparent effect on survival. Pathologic and hematologic examinations not performed.
Rat	50/both	1300	130-139	214	Eye/nose irritation but no effect on weight gain. Rats still often appeared dirty and unkempt. No significant effects on organ weights, gross or histopathology. Hematologic examinations on 18 rats that received 137-139 exposures showed no significant effects.
Guinea pig	12/both	2000	<98	148	Marked eye/nose irritation and weight gain depression. Listlessness and dirty/unkempt appearance. No apparent effect on survival.

TABLE V-3 (cont.)

Species	No./Sex[b]	Approximate Average Vapor Concentration (ppm)	Number of 7-8 hour Exposures[c]	Duration (days)	Effects[d]
Guinea pig	94/both	1300	134-139	214	About 10% mortality after the first few exposures because of histologically verified acute lung irritation. Surviving animals gained weight slowly but showed eye/nose irritation and often appeared dirty and unkempt. The only significant gross or histopathologic effects in the survivors were a few healed pulmonary lesions.
Guinea pig	24/both	650	123-130	189	No effect on condition, weight gain, organ weights or gross pathology.
Rabbit	2/both	2000	<126	148	No apparent irritation or effect on weight gain or appearance (pathologic and hematologic examinations not performed).
Rabbit	12/both	1300	68-264	360	No adverse effects on weight gain, appearance, gross or histolopathology, or hematology. No ophthalmologic effects after 211-226 exposures.

TABLE V-3 (cont.)

Species	No./Sex[b]	Approximate Average Vapor Concentration (ppm)	Number of 7-8 hour Exposures[c]	Duration (days)	Effects[d]
Monkey	2/female	1300	262-264	12 months	No evidence of irritation, intoxication, gross or microscopic pathologic effects, or hematologic effects. An opthalmologic examination of 1 female monkey was unremarkable.
	2/male	1300	142	7 months	

[a] Source: Spencer et al., 1942; Wolf et al., 1956

[b] Number-, sex- and age-matched groups were employed.

[c] Exposures were 5 days/week.

[d] Control groups were employed but not specifically characterized. "Many" of the animals were examined microscopically; lung, liver, kidney, spleen, pancreas, adrenals were routinely examined. "In many cases" other tissues such as the heart, muscle, bone marrow, testis, bladder, optic nerve, sciatic nerve, stomach and intestines were also examined. Hematologic examinations on a "representative group" of rats and rabbits and all monkeys apparently included total erythrocyte and leukocyte counts, differential counts and determination of hemoglobin concentration.

NR = Not reported

to 1300 ppm, apparently caused by severe lung irritation. Rabbits showed no treatment-related effects at 1300 ppm and guinea pigs showed no adverse effects at 650 ppm. No evidence of toxicity was noted in monkeys exposed to 1300 ppm styrene for ~7 months (2 males) or 1 year (2 females).

Other subchronic inhalation studies are summarized in Table V-4. Vainio et al. (1979) exposed male Wistar rats to 300 ppm styrene, 6 hours/day, 5 days/week for ≤11 weeks. Lung glutathione levels were lowered initially but then returned to normal. Liver glutathione levels were significantly depressed after 2 weeks, with some adaptation during longer exposures. The mixed function oxidase system (measured as cytochrome P-450 content and ethoxycoumarin deethylase activity) was increased in the liver and kidney beginning at 2 weeks. A significant increase in UDP glucuronosyl transferase activity occurred later (at 6 weeks) in both the liver and kidneys. Hepatic epoxide hydratase was elevated by the end of the study. Histological liver alterations (parenchymal hydropic degeneration, steatosis and congestion) were found after as little as 2 of weeks of exposure.

After inhalation exposure of rats to styrene (450 ppm, 8 hours/day for 7 days), UDP glucuronosyl transferase and epoxide hydratase activity increased significantly only in the liver (not kidneys or lungs) and ethoxycoumarin deethylase activity increased significantly only in the kidneys (Sandell et al., 1978a,b). No significant changes were observed in the activity of benzpyrene hydroxylase, NADPH cytochrome _c_-reductase, cytochrome P-450 or glutathione-S-transferase in the liver, kidneys and lungs.

TABLE V-4

Other Subchronic Inhalation Studies of Styrene

Species/Strain	Sex/No.	Age or Weight	Concentration (ppm)	Exposures	Duration	Effects	Reference
Rat/Wistar	male 40	adult	300	6 hours/day 5 days/week	11 weeks	Lung glutathione levels were lower ($p<0.05$) after 2 and 4 weeks of exposure but not after 6 and 11 weeks. Liver glutathione levels significantly ($p<0.001$) depressed after 2 weeks with some adaptation during longer exposures. Hepatic cytochrome P-450 content and ethoxycoumarin deethylase activity increased significantly ($p<0.01$) within 2 weeks and remained elevated. Activity of hepatic glucuronosyl transferase was elevated by 6 weeks ($p<0.05$), and epoxide hydratase was elevated by the end of the study. Renal cytochrome P-450 content significantly ($p<0.05$) increased only at 11 weeks. Renal UDP glucuronosyl transferase activity significantly ($p<0.01$) increased from 6 weeks+, ethoxycoumarin deethylase activity significantly ($p<0.001$) increased from 2 weeks+. Parenchymal hydropic degeneration, steatosis and congestion in livers after 2 weeks+ styrene.	Vainio et al., 1979
Rat/Wistar	male 4-8/assay	~300 g	450	8 hours/day	7 days	No change in activity of benzpyrene hydroxylase, NADPH cytochrome *c*-reductase, cytochrome P-450 or glutathione-*S*-transferase in liver, kidneys or lungs. Ethoxycoumarin deethylase activity increased significantly in the kidneys; UDP glucuronosyl transferase and epoxide hydratase activity increased significantly in the liver.	Sandell et al., 1978a,b

TABLE V-4 (cont.)

Species/Strain	Sex/No.	Age or Weight	Concentration (ppm)	Exposures	Duration	Effects	Reference
Rat/Wistar	male 40	adult	300	6 hours/day 5 days/week	2-11 weeks	Statistically significant changes ($p<0.05$-0.0025): Increased serum creatine kinase activity at 4 and 6 weeks, decreased cholinesterase at 2 and 4 weeks. Brain: increased activities of creatine kinase and acetylcholinesterase after 9 weeks, increased acid proteinase activity at weeks 9 and 11, decreased protein content at weeks 9 and 11, increased RNA at week 11. Minor alterations in protein composition of spinal cord axons at weeks 9 and 11.	Savolainen and Pfaffli, 1977
Rat/Wistar	male 26	adult	300	6 hours/day 5 days/week	4-17 weeks	Results 4, 8, 13 and 17 weeks: No significant effect on glial glutathione concentration or NADPH-diaphorase activity. Significant ($p<0.05$) decrease in glial acid proteinase only at 4 weeks. Behavior (ambulation, rearing, preening frequency, preening time) was not altered.	Savolainen et al., 1980
Rat/NR	NR 20 15 control	young adult	300	6 hours/day 5 days/week	11 weeks	Motor conduction velocity of tail nerve significantly higher ($p<0.05$) after 6 weeks but not after 8 or 11 weeks.	Seppalainen, 1978
Rat/Sprague-Dawley	female 96/group	7-8 weeks	0, 600, 1000*	6 hours/day 5 days/week	20.7 months	Mean body weights significantly decreased for 1st 506 days in 1000 ppm group. Mean liver weights and liver/body weight ratios were significantly higher for 600 ppm at 6 and 12 months and for 1000 ppm at 6 and 24 months.	Jersey et al., 1978

TABLE V-4 (cont.)

Species/Strain	Sex/No.	Age or Weight	Concentration (ppm)	Exposures	Duration	Effects	Reference
Rat/Sprague-Dawley	female 96/group	7-8 weeks	0, 600, 1000*	6 hours/day 5 days/week	20.7 months	Hematology, urinalysis and clinical chemistry results did not indicate any exposure-related changes. The only gross and histopathologic changes at termination of study (24 months) considered possibly exposure-related were significant increases in small subpleural pale foci in the lungs (both exposures) and focal alveolar histiocytosis (1000 ppm).	Jersey et al., 1978
Rat/Sprague-Dawley	male 96/group	7-8 weeks	0, 600, 1000*	6 hours/day 5 days/week	18.3 months	High incidence of murine pneumonia in control and treated groups affected results of study. Mean body weights were significantly decreased in treated rats for 1st 263 days; 600 ppm group then had weights similar to control. No significant differences in hematology, urinalysis and clinical chemistry results. Rats necropsied at 6 months had statistically significant decreases in absolute mean weights of kidney (both exposures) and heart and liver (1000 ppm); at 12 months there were significant decreases in mean liver (both exposures) and kidney (1000 ppm) weights. After 6 and 12 months a minimal decrease in the cytoplasmic microvesiculation of the hepatocytes was observed indicative of a depressed nutritional state. 2-year necropsy: None of the statistically significant histopathologic changes were considered exposure-related.	Jersey et al., 1978

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*1200 ppm for first 2 months

NR = Not reported

Savolainen and Pfaffli (1977) exposed rats to 0 or 300 ppm styrene 6 hours/day, 5 days/week for 2, 4 and 6-11 weeks. Styrene levels in the brain and perinephric fat peaked at 4 weeks and, then declined progressively. Serum creatine kinase activity increased at 4 and 6 weeks and nonspecific cholinesterase activity decreased during the first few weeks. In the brain, activities of these enzymes (creatine kinase and acetylcholinesterase) increased significantly only after 9 weeks of exposure. Other biochemical alterations detected in the brain near the end of the 11th week of exposure included increased acid proteinase activity, decreased protein content and increased RNA content. Electrophoresis showed minor alterations in the protein composition of spinal cord axons (from the 9th week on) but not in water soluble cerebellar fractions. The authors concluded that the transient serum enzyme changes reflected metabolic adaptation and the biochemical changes in the brain may be an early indication of neurotoxic action.

In a similarly designed study, Savolainen et al. (1980) found little change in the concentration of glial acid proteinase (significant decrease at 4 weeks only). No significant changes were observed in glutathione concentration or NADPH-diaphorase activity in glial cells. These results, indicating that styrene had almost no effect on glial cells, were interesting (Savolainen et al., 1980), since protein destruction was evident in whole brain homogenate after 9 weeks of exposure in the study cited above (Savolainen and Pfaffli, 1977). Behavioral changes were not observed. The interaction of styrene and ethanol was also studied by substituting 15% ethanol for drinking water (Savolainen et al., 1980). Acid proteinase activity increased significantly in the ethanol-dose group, but co-exposure to styrene resulted in a significantly damped effect at weeks 4 and 13. Increased preening time at week 4 and increased ambulation and rearing at week 13 in the styrene and ethanol group suggested a possible interaction of styrene and ethanol.

A transient increase in the motor conduction velocity of the tail nerve was observed in rats exposed to 300 ppm styrene for 6 hours/day, 5 days/week for ≤11 weeks (Seppalainen, 1978). The author noted that this increase may indicate beginning neurotoxicity, but no further supporting data were provided.

Jersey et al. (1978) conducted a chronic inhalation study of styrene (groups of 96 rats were exposed to 0, 600 or 1000 ppm styrene, 6 hours/day, 5 days/week). The high-dose group was exposed to 1200 ppm styrene originally, but the concentration was reduced to 1000 ppm because overt narcosis became evident in males. Exposures were terminated when 50% mortality was reached in one group of each sex. Surviving animals were sacrificed at 24 months. In females, there was a significantly reduced mean body weight at the high-dose and increased liver weight at both doses at certain measurement times. Lung effects were noted at the termination of the study (Table V-4), but no other gross or histopathologic changes were considered exposure-related. In males, the results are difficult to interpret because of the high incidence of murine pneumonia (not exposure-related). A depressed nutritional state was indicated by decreased mean body weight and a minimal decrease in the cytoplasmic microvesiculation of the hepatocytes.

Dermal Exposure. Twenty applications of undiluted styrene (volume not stated) to the ear of a rabbit over a 4-week period caused moderate irritation with blistering and hair loss, but no systemic toxic effects were noted (Spencer et al., 1942; Wolf et al., 1956). Additional details of this study were not provided.

Target Organ Toxicity

Liver. Following acute exposure, a significant ($p<0.05$) decrease in hepatic glutathione content was reported in rats (Vainio and Makinen, 1977; Das et al., 1981a), isolated rat hepatocytes (Zitting et al., 1980), hamsters (Vainio and Makinen, 1977; Parkii et al., 1978), mice (Vainio and Makinen, 1977) and guinea pigs (Vainio and Makinen, 1977). Glutathione depletion in rats occurred within 2 weeks of initiation of inhalation exposure (300 ppm x 6 hours/day) (Vainio et al., 1979) and following oral dosing with 900 mg styrene/kg/day for 7 days (no depletion at 450 mg/kg/day) (Das et al., 1981a). Glutathione-S-transferase activity was depressed at 450 and 900 mg/kg/day (Das et al., 1981a). Srivastava et al. (1982) also noted a dose-dependent decrease in the activity of glutathione-S-transferase after subchronic oral dosing.

Epoxide hydrase activity increased significantly in rats after intraperitoneal injection of styrene (Parkki et al., 1976) and following 7-day inhalation exposure to 450 ppm 8 hours/day (Sandell et al., 1978a,b). Vainio et al. (1979) found that epoxide hydratase and UDP glucuronosyl transferase activity increased gradually in rats over 11 weeks of inhalation exposure (300 ppm x 6 hours/day). Although the activity of one enzyme (glutathione-S-transferase) that can act to detoxify the styrene metabolite, styrene oxide, appears to be decreased after exposure to styrene; another detoxifying enzyme, epoxide hydrase, appears to increase its activity following styrene exposure.

Induction of MFO has been observed following exposure to styrene. Das et al. (1981a) found significant increased activities of aminopyrine-N-demethylase, ethylmorphine-N-demethylase and aniline hydroxylase at oral doses of 450 and 900 mg/kg/day for 7 days, but not at a dose level of 270 mg/kg/day. Cytochrome P-450 content and ethoxycoumarin deethylase activity increased in rats within 2 weeks of initiation of inhalation exposure (Vainio et al., 1979). Aryl hydrocarbon hydroxylase activity decreased in rats after a single intraperitoneal dose of styrene (Roberfroid et al., 1978) but increased after 7 days of oral dosing (450 and 900 mg/kg) (Das et al., 1981a).

Mitochondrial succinic dehydrogenase and beta-glucuronidase activities decreased at both 200 and 400 mg/kg in a subchronic study, while acid phosphatase activity decreased only at the higher dose (Srivastava et al., 1982).

Lambotte-Vandepaer et al. (1979) studied the effect of single intraperitoneal doses of styrene on the substrate affinity (Km) and activity (Vmax) of several hepatic enzymes in rats. The Vmax of benzo(a)pyrene hydroxylase (a typical aryl hydrocarbon hydroxylase enzyme) was not significantly altered, but the Km value decreased rapidly for ~24 hours, remained low at 48 hours and then returned to normal. Styrene oxide hydratase had a decreased Km value (increased affinity for styrene oxide) within 6 hours after styrene administration, and it remained low until 48 hours postinjection. The Vmax of styrene oxide hydratase did not change. The Vmax and Km values were not significantly altered at 24 hours for styrene epoxidase.

Histological alterations consisting of parenchymal hydropic degeneration, steatosis and congestion were found in rat livers after 2-week inhalation exposure to 300 ppm 6 hours/day, 5 days/week (Vainio et al., 1979). Minimal histopathological changes including increasing iron deposits within the reticuloendothelial cells and increased numbers of intranuclear acidophilic crystalline inclusions on hepatocytes were observed in dogs following subchronic oral exposure to styrene (600 mg/kg/day for 560 days) (Quast et al., 1978). Tiny areas of focal necrosis were observed in the liver following subchronic oral dosing of rats (400 mg/kg/day) (Srivastava et al., 1982). The glycogen content was reduced in the liver after intragastric administration of styrene (Delag et al., 1976).

Several investigators have noted increased serum levels of enzymes that are commonly used as indicators of liver dysfunction following styrene exposure. Chakrabarti and Brodeur (1981b) noted increased activities in SGOT and SGPT in rats after intraperitoneal injection of 878 mg styrene/kg. Increases in the activity of SGOT and SGPT were also observed in rat serum enzyme assays at the high dose (400 mg/kg/day) in an oral subchronic study (Srivastava et al., 1982).

Chakrabarti and Brodeur (1981a) reported that biliary excretion of bromosulfophthalein was significantly ($p<0.05$) reduced for 30, 45 and 65 minutes in rats pretreated with 234, 468 and 908 mg styrene/kg.

Styrene oxide causes alterations similar to styrene but the effects are more pronounced. Chakrabarti and Brodeur (1981a) found that bile flow was depressed to ~50% of the control for the entire 90-minute collection period after intraperitoneal injection of styrene oxide in rats, and biliary excretion of bromosulfophthalein was similarly depressed.

Depletion of glutathione occurred almost immediately after inhalation exposure to styrene oxide; the level gradually returned to normal (Vainio and Elovaara, 1979). Within 2 hours of intraperitoneal injection of styrene oxide (375 mg/kg) hepatic glutathione levels in rats were reduced to ~12% of control levels (Chakrabarti and Brodeur, 1981c). In contrast, glutathione levels were not affected in hamsters after intraperitoneal injection of styrene oxide ($\leq$1000 mg/kg) (Parkki et al., 1978), indicating possible interspecies variation in metabolic pathways.

Styrene oxide was found to bind to hepatic microsomal cytochrome P-450 (Vainio and Elovaara, 1979). Parkki et al. (1976) noted significant decreases in benzypyrene hydroxylase and p-nitroanisole o-demethylase activation and cytochrome P-450 content after intraperitoneal injection of styrene oxide in rats but epoxide hydratase, UDP glucuronosyl transferase and NADPH cytochrome-C reductase activities were not changed significantly. Klinman et al. (1977) reported styrene oxide inhibited horse liver alcohol dehydrogenase by direct binding and alkylation at two cystein residues.

Kidney. Changes in renal enzyme activities have been observed in rats after exposure to styrene. UDP glucuronosyl transferase and ethoxycoumarin deethylase activity increased significantly in subchronic inhalation studies

(Vainio et al., 1979; Sandell et al., 1978a,b). Cytochrome P-450 content increased significantly after 11 weeks of inhalation exposure (Vainio et al., 1979). After oral dosing for 7 days, the activity of aryl hydrocarbon hydroxylase, aniline hydroxylase, benzphetamine-N-demethylase and ethylmorphine-N-demethylase increased significantly, while glutathione-S-transferase activity was significantly decreased (Das et al., 1983). Renal glutathione content decreased significantly only at the higher dose (900 mg/kg) (Das et al., 1983).

Nervous System and Sensory Organs. Savolainen and Pfaffli (1977) studied the biochemical alterations in the brain after subchronic inhalation exposure of rats to styrene at 300 ppm. A number of statistically significant ($p<0.05-0.0025$) alterations were evident after ≥9 weeks of exposure including increased creatine kinase, acetylcholinesterase and acid proteinase activities, decreased protein content and increased RNA content. There were simultaneous minor alterations in the protein composition of spinal cord axons but not in water soluble cerebellar fractions. Savolainen et al. (1980) subsequently studied biochemical changes in cerebral glial cells. The glial acid proteinase concentration was affected minimally by styrene exposure. Glial glutathione concentration was not affected. Behavior (ambulation, rearing, preening frequency, preening time) was also unaffected by styrene exposure.

Husain et al. (1980) found brain monoamine oxidase activity decreased, but acetylcholinesterase was unchanged after a 15-day oral exposure of 1.0 mℓ styrene/kg bw. Glutathione-S-transferase activity decreased in the brain after 7 days of oral dosing with styrene at either 450 or 900 mg/kg bw/day in groundnut oil (Das et al., 1981b); brain glutathione and aryl hydrocarbon hydroxylase activity levels were also depressed after similar treatment (Dixit et al., 1982).

Levels of brain noradrenaline and serotonin increased significantly after a 15-day oral exposure (as above), but dopamine levels were unchanged (Husain et al., 1980). A significant increase in the binding of ^{3}H-spiroperidol to dopamine receptors in the corpus straitum occurred in rats after single oral doses and repeated doses for 90 days (Agrawal et al., 1982).

A transient increase in the motor conduction velocity of the tail nerve of rats was observed during a subchronic inhalation study (Seppalainen, 1978).

Increased swimming activity was observed during a 3-minute behavioral swimming test after a 4-hour inhalation exposure to styrene (De Ceaurriz et al., 1983). Mice exposed to 801 and 851 ppm were immobile for an average of only 24 and 14 seconds, respectively, while their matched controls were immobile for 106 and 79 seconds during the 3-minute test.

Intravenous administration of styrene producing a blood concentration of 40 ppm produced vestibular disturbances determined by position nystagmus in rats (Tham et al., 1982) and rabbits (Larsby et al., 1978; Odkvist et al., 1979, 1980). Intravenous doses of styrene as low as 3 mg/kg produced amplitude increases of the C-wave and increases in the standing potential of the eye of cynomolgus monkeys following light stimulation (Skoog and Nilsson, 1981).

Other Effects

Reproductive and Developmental. Styrene has been studied for teratogenic effects in rats by oral exposure and in rats, mice, rabbits and hamsters by inhalation exposure. The results of these studies are summarized in Table V-5.

Beliles et al. (1985), in combination with chronic toxicity, studied reproductive performance through three generations of Sprague-Dawley rats treated with styrene in their drinking water at nominal concentrations of 0, 125 and 250 ppm. Using food and water consumption data corresponding doses were 7.7 and 14 mg/kg/day for males and 12 and 21 mg/kg/day for females. After animals were dosed for 90 days, 20 females and 10 males from the styrene groups and 30 female and 15 males from the control group were used as F_0 generation breeders. Pups, designated F_1 generation, were treated until they were 110 days of age. These animales were then mated and the offspring were designated F_2 generation. A third generation (F_3) was produced in the same manner. Each generation was evaluated for fertility, litter size, pup viability, pup survival, sex ratio, pup body weight, weanling liver and kidney weight, and marrow cytogenetics. The authors concluded that all the data evaluated for reproductive performance showed no evidence of styrene-related changes.

Investigators at the Dow Chemical Company administered styrene to pregnant Sprague-Dawley rats by gavage (in peanut oil) at dose levels of 0 (vehicle control), 180 or 300 mg/kg/day (0, 90 or 150 mg/kg twice daily) on days 6-15 of gestation (Murray et al., 1976, 1978a). Maternal toxicity was indicated by significantly reduced ($p<0.05$) body weight gain and food

TABLE V-5

Reproductive and Developmental Toxicity of Styrene

Route	Species/ Strain	No. Dams at Start	Concentration or Dose	Exposure Duration	Maternotoxic, Fetotoxic and Developmental Effects	Reference
Oral (gavage)	rats/Sprague-Dawley	29-39/group	0, 180, 300 mg/kg/day	days 6-15 of gestation	Significantly ($p<0.05$) decreased maternal body weight gain and decreased food consumption on days 6-9 of gestation at both dose levels. Maternal mortality and percent pregnancy were not affected significantly. No significant treatment-related effects on mean incidences of implantations/dam, live fetuses/litter or resorption/litter, or on mean fetal body weight or crown-rump length. Gross external, soft-tissue and skeletal examinations did not reveal any teratogenic effects. Number of litters examined, 32, 24 and 24 in 0, 180 and 300 mg/kg group; 1/3 of the fetuses of each litter were examined for soft-tissue alterations.	**Murray et al., 1976, 1978a**
Oral (drinking water)	rats/Sprague-Dawley	20-30/group	0, 125 and 250 ppm	90-110 days	Three generations of offspring maintained on styrene drinking water showed no reproductive changes when fertility, litter size, pup viability, pup survival, sex ratio, pup body weight, weanling liver and kidney weight, and marrow cytogenetics were evaluated.	**Beliles et al., 1985**
Inhalation	rats/Sprague-Dawley	29-30/group	0, 300, 600 ppm	7 hours/day days 6-15 of gestation	Reduced maternal body weight gain and decreased food consumption on days 6-9 of gestation at both exposures. Number of live fetuses, resorptions/litter, and mean fetal body weight not significantly affected. Average fetal crown-rump length decreased significantly only at 300 ppm. The incidence of skeletal variations (lumbar spurs, delayed ossification of sternebrae and vertebral centra) was significantly higher in the 300 ppm group only, but the incidences were consistent with the range of incidences in historical controls. No significant differences were observed in the incidences of external and visceral malformations.	**Murray et al., 1978a,b**

TABLE V-5 (cont.)

Route	Species/ Strain	No. Dams at Start	Concentration or Dose	Exposure Duration	Maternotoxic, Fetotoxic and Developmental Effects	Reference
Inhalation	rats/NR	NR	0.35, 1.2 and 12 ppm	4 hours/day throughout gestation	Resorption noted at all exposure levels. No significant increase in the number of malformations. No additional details are available; the primary report of this Russian study was not obtained.	Ragule, 1974
Inhalation	mice/BMR/1616	NR	0, 250 ppm	6 hours/day days 6-16 of gestation	Embryotoxicity was indicated by increased (p<0.1) incidences of dead or resorbed fetuses (28/104 or 27%) when compared with the control (21/115 or 18%). The number of live fetuses/litter was not significantly altered. No teratogenic effects were observed. A higher percentage of fetuses with minor skeletal abnormalities (rib fusions and extra ribs) occurred in the treated group (2.9% in 104 fetuses from treated litters, 0.9% in 115 fetuses from control litters).	Kankaanpaa et al., 1980
Inhalation	hamsters/ Chinese	NR	0, 300, 500, 750, 1000 ppm	6 hours/day days 6-18 of gestation	A significantly (p<0.001) greater number of dead or resorbed fetuses was observed at 1000 ppm (33/50) than at 0 ppm (28/107). The number of live fetuses/litter at 1000 ppm (2.4) was not significantly different from the control (5.3). No malformations were reported in any of the hamsters. The number of fetuses examined were 107, 14, 23, 38 and 50 at 0, 300, 500, 750 and 1000 ppm, respectively.	Kankaanpaa et al., 1980
Inhalation	rabbits/ New Zealand	20/group	0, 300, 600 ppm	7 hours/day days 6-18 of gestation	No significant effects were observed on maternal toxicity. No significant effects at either exposure level on the mean incidence of implantation/litter, live fetuses/litter or resorptions/litter, or mean fetal body weight or crown-rump length. Gross external and cleft palate examinations of all fetuses and gross visceral examinations of 1/3 of the fetuses/litter revealed no malformed fetuses in any groups. There was a significant (p=0.049) increase in the incidence of unossified fifth sternebrae among litters of the 600 ppm group, but the incidence was reportedly within the range of historical controls.	Murray et al., 1978a,b

NR - Not reported

consumption on days 6-9 of gestation, but no significant effects were observed on maternal mortality or percent pregnancy. No teratogenic or fetotoxic effects were observed. Dow Chemical Company also conducted a teratologic evaluation of styrene administered to rats by inhalation (Murray et al., 1978a,b). Pregnant Sprague-Dawley rats were exposed to 0 or 300 ppm of styrene 7 hours/day for days 6-15 of gestation. Additional rats were subsequently exposed using the same protocol to 0 or 600 ppm of styrene. Results similar to the oral study were obtained for the maternal toxicity indices. Decreased body weight gain and decreased food consumption on days 6-9 of gestation were observed in both treatment groups, but maternal mortality and percent pregnancy were not significantly altered. The average fetal crown-rump length was significantly reduced in the 300 ppm group, but not in the 600 ppm group, so this effect may not be treatment related. There was a significantly greater incidence of a few skeletal variants in the 300 ppm group (observed in 3/24 litters), but the incidences observed were similar to historical controls. Murray et al. (1978a) found that styrene did not significantly affect numbers of live fetuses or resorptions/litter, whereas Ragule (1974) reported an increased incidence of resorptions among rats exposed to a much lower concentration (0.35-10 ppm of styrene for 4 hours/day throughout pregnancy). An explanation for this difference is not evident, but Murray et al. (1978a) noted that Ragule (1974) reported an absence of resorption in the control rats, which is highly unusual, suggesting that the controls may not be representative.

Embryotoxicity was indicated following exposure of pregnant mice to 250 ppm styrene 6 hours/day for days 6-16 of gestation (Kankaanpan et al., 1980). The incidence of dead or resorbed fetuses was higher ($p<0.10$) in the exposed group (27%) as compared with the control group (18%). The number of live fetuses/litter was not significantly altered. The number of dead or resorbed fetuses in preliminary studies at doses of 500 and 750 ppm (which resulted in maternal deaths of 2/6 and 3/5 mice, respectively) was 22/47 and 19/20, respectively. A higher percentage (not significantly different) of fetuses with minor skeletal abnormalities was reportedly observed in the treated group, but no teratogenic effects were observed.

Fetotoxicity was also indicated when Chinese hamsters were exposed to 1000 ppm styrene 6 hours/day from days 6-18 of gestation (Kankaanpaa et al., 1980). A significantly ($p<0.001$) greater number of dead or resorbed fetuses was observed compared with controls, although the number of live fetuses/litter did not differ significantly (2.4 compared with 5.3 for controls). Fetotoxicity was not observed at exposure levels of 300, 500 or 750 ppm. Teratogenicity was not reported at any exposure level.

Maternal toxicity was not observed in rabbits exposed to 300 or 600 ppm of styrene on days 6-18 of gestation, which contrasts to rats exposed on days 6-15 of gestation (Murray et al., 1978a,b). No fetotoxic or teratogenic effects were observed in rabbits. There was a statistically significant increase ($p=0.049$) in the incidence of unossified fifth sternebrae in the 600 ppm group, but the incidence was reportedly within the range normally observed in recent studies at that laboratory.

The reproductive/teratogenic effects of styrene oxide were assessed in Wistar rats and New Zealand white rabbits in an inhalation study performed by Battelle Pacific Northwest Laboratory for NIOSH (Sikov et al., 1981;

Hardin et al., 1981). There were four groups of rats exposed to either filtered air or 100 ppm styrene oxide for 3 weeks before gestation with subsequent exposure to either filtered air or 100 ppm styrene oxide for days 1-19 of gestation. The groups were designated as air-air (control), air-styrene oxide (48 rats exposed to styrene oxide during gestation), styrene oxide-air (32 rats with pregestational exposure to styrene oxide) and styrene oxide-styrene oxide (31 rats exposed to styrene oxide before and during gestation). Maternotoxic effects were observed including significantly ($p<0.05$) reduced mean body weight gain and food consumption in all treatment groups during most measurement periods, and in the air-styrene oxide and styrene oxide-styrene oxide groups increased lung and kidney-to-body weight ratios and treatment-related bronchiolitis and bronchopneumonia. Percent pregnancy was significantly ($p<0.05$) reduced in the air-styrene oxide group (12/39) and the styrene oxide-styrene oxide group (5/28) compared with most quantitative indices of reproductive success, but the number of litters was low in the treated group. Fetal toxicity was indicated by a reduction in fetal body and crown-rump length in the groups exposed during gestation. These reductions were significant ($p<0.05$) in the air-styrene oxide group. There was no indication of frank teratogenic effects.

The inhalation study with rabbits (Sikov et al., 1981; Hardin et al., 1981) involved exposure of groups of 23-24 rabbits to 0, 15 or 50 ppm styrene oxide for 7 hours/day for days 1-24 of gestation. There was significantly increased maternal mortality (19/24 vs. 1/23 in control) and reduced body weight gain in the high-dose group. Percent pregnancy was not significantly affected, but the percentage of litters with resorptions was significantly ($p<0.05$) increased in both groups of treated rabbits (based on only four litters in the high-dose group because of maternal mortality). No teratogenic effects were observed.

Mutagenicity. The mutagenic potential of styrene and some of its metabolites has been studied extensively in the reverse mutation assay developed by Ames et al. (1973). The studies testing styrene for mutagenicity in Salmonella typhimurium are summarized in Table V-6.

There was no increase in the number of revertant colonies of S. typhimurium when styrene was assayed in the absence of a mammalian metabolic activating system (S-9 fraction) (see Table V-6). There were mixed positive and negative results when styrene was assayed in the presence of a metabolic activating system derived from Aroclor-induced rat liver (S-9) (see Table V-6). Negative results were obtained using bacterial strains TA1535, TA1537, TA98 and TA100 in both plate incorporation and spot test procedures at doses of styrene up to 1 mg/plate, which was toxic to the bacteria (Stoltz and Withey, 1977; De Flora, 1981; Busk, 1979; Loprieno et al., 1978). Simmon et al. (1977) also obtained negative results following vapor exposure. De Meester et al. (1977, 1981) and Vainio et al. (1976) obtained positive results with mutant strains sensitive to base pair substitution, while all tests were negative in strains sensitive to frameshift mutagens. A 10-fold increase in the number of revertants was the maximum that occurred over a narrow dose range of ~2-11 μmole/plate (De Meester et al., 1977). Only 5% of the cells survived the highest dose. Vainio et al. (1976) suggested the high toxicity of styrene made interpretation of the results difficult.

TABLE V-6

Mutagenicity Assays of Styrene in Salmonella typhimurium

Exposure Method	Dose Level	Metabolic Activation*	Tester Strain	Response	Comments	Reference
spot test	NR	+/-	TA1535 TA100 TA1537 TA1538 TA98	-/- -/- -/- -/- -/-		Busk, 1979
spot test	5 µl		TA1535 TA100 TA1537 TA1538 TA98	- - - - -		Milvy and Garro, 1976
plate incorporation	up to 5 µmol/plate (up to 0.52 mg/plate)	+/-	TA1535 TA1537 TA1538 TA98 TA100	-/ -/ -/ -/ -/	The maximum dose tested was toxic.	de Flora, 1981
plate incorporation	up to 1 mg/plate	+/	TA1535 TA1537 TA1538 TA98 TA100	-/ -/- -/ -/ -/-	The assays were performed with both rat liver and hamster derived S-9. In addition to the plate incorporation assay, a spot test was conducted with similar negative results.	Stoltz and Withey, 1977
plate incorporation	0.001-10 µmol/plate (0.0001-1.0 mg/plate)	+/-	TA1535	-/-	Metabolic activation was provided by liver preparations from male CD1 mice pretreated with phenobarbital.	Loprieno et al., 1978
plate incorporation	0.001-15 µmol/plate (0.001-1.6 mg/plate)	+/-	TA1535 TA100	-/- -/	At 15 µmol/plate, only 1% of the cells survived. The S-9 was prepared from both Aroclor 1254 and Clophen-C pretreated rats. Inhibitors of epoxide hydratase and glutathione conjugation was included in the assay.	Busk, 1979

TABLE V-6 (cont.)

Exposure Method	Dose Level	Metabolic Activation*	Tester Strain	Response	Comments	Reference
plate incorporation	1-15 µmol/plate (0.104-1.56 mg/plate)	+/-	TA1535 TA1537 TA1538 TA98 TA100	+/- -/- -/ -/- -/	In the absence of S-9, the highest dose tested was 100 µmol/plate with ~10 times the spontaneous level reached at 11 µmol/plate. Survival at 11 µmol/plate was only 12%.	De Meester et al., 1977
plate incorporation	0.01-100 µmol/plate (0.001-10.4 mg/plate)	+/-	TA1535 TA1537 TA1538 TA100 TA98	+/- -/- -/- +/- -/-	Styrene produced a > doubling of the spontaneous rate at a single dose 10 and 1 µmol/plate for strains TA1535 and TA100, respectively. A more detailed study of TA1535 confirmed that styrene had a narrow effective range.	Vainio et al., 1976
plate incorporation	10 µmol/plate	+	TA1535	+	Styrene was also studied by liquid incubation atmospheric exposure and the fluctuation test. Negative results were obtained with all but the plate incorporation test where there was approximately a 10-fold increase in revertant numbers.	Poncelet et al., 1980
atmospheric exposure	NR	+	TA100 TA1535 TA1537 TA98	- - - -	Styrene was listed as 1 of 45 nonmutagenic compounds in this survey of the mutagenic potential of 71 chemicals identified in drinking water.	Simmon et al., 1977
atmospheric exposure	24% (v/v) for 24 hours (240,000 ppm)	+/-	TA1530 TA1535 TA100 TA98 TA1538 TA1537 G46	+/- +/- +/- -/- -/- -/- -/-	An approximate 2- to 4-fold increase over the spontaneous rate was observed in the responsive strains.	De Meester et al., 1981

*Provided by S-9 derived from rat liver unless otherwise specified in the comment.

NR = Not reported

In a review of the results of 10 studies in which styrene was tested for mutagenicity in Salmonella typhimurium, Kier et al. (1986) found that styrene was positive with activation and negative without. Kier et al. (1986) concluded, however, that these studies provided limited evidence of mutagenicity due to the high cytotoxicity of styrene.

Styrene oxide, a major metabolite of styrene, has consistently been demonstrated to be a mutagen both in the presence and absence of a mammalian metabolic activation system in S. typhimurium strains TA1535 and TA100 (De Meester et al., 1977, 1981; Busk, 1979; De Flora, 1981; Glatt et al., 1975; Loprieno et al., 1978; Milvy and Garro, 1976; Vainio et al., 1976; Drinkwater et al., 1978). No mutagenic activity was observed with any of the tester strains sensitive to frameshift mutagens.

Watabe et al. (1982b) tested the mutagenicity of 1-vinylbenzene-3,4-oxide, another presumed metabolite of styrene. Only weak mutagenic activity was observed when tested by the preincubation procedure using S. typhimurium strain TA100, compared with styrene oxide activity of 1053 revertants/10 µmoles/plate (1.04 mg/plate). The compounds were then tested by adding 25% of the total dose to the incubation mixture at 5 minute intervals because the arene oxide was found to be unstable in aqueous buffer. 1-Vinylbenzene-3,4-oxide was much more mutagenic under these conditions [~8000 revertants/0.2 µmoles/plate (0.0024 mg/plate)], while the activity of styrene oxide was similar to the previous results.

The styrene metabolite, styrene glycol, was nonmutagenic when tested in bacterial strains TA1535, TA1537, TA1538, TA98 and TA100 with and without metabolic activation (De Flora, 1981; Vainio et al., 1976). Mercapturic acid precursor metabolites of styrene were also nonmutagenic in strain TA100 (Pagano et al., 1982).

Since some metabolic intermediates of styrene were mutagenic in S. typhimurium, but styrene demonstrated only limited mutagenic activity, several studies have been conducted to investigate the effect of *in vitro* metabolism of styrene by the S-9 fraction on the formation of mutagenic metabolites. Styrene was weakly mutagenic toward S. typhimurium strain TA100 in the presence of the epoxide hydratase inhibitor TCPO and rat liver S-9 fractions fortified with NADPH-generating systems (Watabe et al., 1978b,c). These two reports appeared to contain identical data, which was also summarized in an abstract (Watabe et al., 1979). Since mutagenic activity was not observed when TCPO was omitted, it was inferred that the limited mutagenic activity of styrene resulted from the rapid metabolism of styrene metabolites to nonmutagenic compounds by the action of epoxide hydratase. Chemical analyses, however, indicated styrene oxide levels were not sufficient to account for the mutagenic response, which led the authors to propose that another styrene metabolite (possibly 1-vinylbenzene-3,4-1-vinylbenzene-3,4-oxide) might have contributed to the positive response.

Yoshikawa et al. (1980) found a marked decrease in the mutagenic activity of styrene oxide when gluatnione was added in the presence of a soluble supernatant fraction, which indicated that glutathione was reacting with styrene oxide through a component of the soluble fraction (presumably glutathione-S-transferase) to produce a nonmutagenic compound. El-Tantawy and Hammock (1980) concluded from their results that epoxide hydratase (not glutathione-S-transferase) was the primary enzyme involved in the inactivation of styrene oxide.

The results of mutagenicity assays of styrene in eukaryotic organisms (yeast *in vivo* and *in vitro*, mammalian cells *in vitro*, *Drosophila melanogaster in vivo*) are summarized in Table V-7. No increases were observed in forward mutation in the yeast, *Schizosaccharomyces pombe*, in the presence or absence of metabolic activation (Bauer et al., 1980; Loprieno et al., 1976; Loprieno, 1977, 1979). In *Saccharomyces cerevisiae*, no change occurred in gene conversions when a metabolic activation system was provided by purified microsomes from mouse livers, but an increase in gene conversion at the *ade* locus was observed in a host-mediated assay (Loprieno et al., 1976).

Mixed positive and negative results were obtained when styrene was tested for mutagenic activity in mammalian cells in culture. Direct incubation of styrene with the test cells (V-79) with or without metabolic activation gave negative responses (or a very marginal positive response with S-9 added) (Loprieno et al., 1976; Beije and Jenssen, 1982). However, there was a statistically significant increase in the mutation rate when a liver perfusion system was used to provide metabolic activation (Beije and Jenssen, 1982). There was no difference in the response level between cells exposed immediately below the liver and cells exposed downstream (transit time 1.5 minutes), which indicated the active compound was not inactivated rapidly.

Donner et al. (1979) found a significant increase in recessive lethal mutations in styrene-treated *Drosophila melanogaster* as compared with

TABLE V-7

Mutagenicity Assays of Styrene in Eukaryotic Cells/Organisms

Test Organisms	Type of Assay	Exposure Method	Dose Level	Metabolic Activation	Response	Comments	Reference
S. pombe (P_1 strain)	forward mutation	liquid incubation	100 mM (10.4 mg/mℓ)	+/-	-/	The metabolic activation was provided by purified microsomes from mouse liver.	Loprieno et al., 1976
S. pombe (P_1 strain)	forward mutation	liquid incubation	100 mM (10.4 mg/mℓ)	+	-	Fresh microsomes were added every 12 minutes during the 60 minute incubation period. Increased toxicity was observed, but no increase in mutagenicity occurred.	Bauer et al., 1980
S. pombe (P_1 strain)	forward mutation	host-mediated (mouse)	1000 mg/kg by gavage	NA	-	The yeast were injected intraperitoneally and reisolated 3, 6 or 12 hours after treatment	Loprieno et al., 1976
S. cerevisiae (D_4)	gene conversion	host-mediated (mouse)	1000 mg/kg by gavage	NA	-	*ade* mutants appeared to increase with incubation time, with 1.03, 4.23, 4.75 and 5.38 mutants x 10^8 cells observed in cells isolated 0, 1, 3 and 6 hours after exposure.	Loprieno et al., 1976
V79 cells	forward mutation	1 hour in culture	8.5 or 17 mM (0.89 or 1.8 mg/mℓ)	-	-	Cells were examined for the ability to grow in the presence of 8-azaguanine.	Loprieno et al., 1976
V79 cells	forward mutation	4 hours in culture	240-960 μmol/4 mℓ (6.25-25.0 mg/mℓ)	+/-	-/-	Cells were examined for the ability to grow in the presence of 6-thioguanine. In the presence of S-9, there was a marginal positive response when the data from the three replication experiments were combined.	Beije and Jennsen, 1982

TABLE V-7 (cont.)

Test Organisms	Type of Assay	Exposure Method	Dose Level	Metabolic Activation	Response	Comments	Reference
V79 cells HGPRT mutations	forward mutation	4 hours to perfusate	240 and 480 µmol/100 mℓ of perfusion media (0.25 0.50 mg/mℓ)	+	+	Cells were examined for the ability to grow in the presence of 6-thioguanine. The metabolic activation was provided by an isolated perfused rat liver.	Beije and Jenssen, 1982
D. melanogaster	recessive lethal	in diet	200 ppm	NA	+	Induction of metabolism increased the frequency of mutations.	Donner et al., 1979

NA Not applicable

controls, with frequencies of 0.09 and 0.60% observed in the control and styrene-exposed groups, respectively. Induction of drug metabolizing enzymes by pretreatment with phenobarbital resulted in a doubling of the frequency of recessive lethal mutations.

Styrene oxide was mutagenic in most eukaryotic test systems including yeast, specifically *Schizosaccharomyces pombe* without metabolic activation and *Saccharomyces cerevisiae* with and without activation (Loprieno et al., 1976), cultured mammalian cells, specifically V-79 without activation (Loprieno et al., 1976; Sugiura et al., 1979; Beije and Jenssen, 1982) and mouse lymphoma L1578Y cells with activation (Amacher and Turner, 1982) and *Drosophila melanogaster* at exposures of 200 ppm in the diet (Donner et al., 1979) and 200 ppm 4 hours/day for 4 days inhalation (Donner et al., 1979). Negative results were observed for *D. melanogaster* at a lower exposure (100 ppm for 2.5 hour inhalation) (McGregor, 1981), for V-79 cells in the presence of metabolic activation by a liver perfusion system (Beije and Jenssen, 1982), and for the yeast, *S. pombe*, in a host-mediated assay (Loprieno et al., 1976).

Styrene has been tested for the ability to produce chromosomal damage using animal cells *in vitro* and using cells from different organs of experimental animals after *in vivo* exposure to styrene. The results of these studies are summarized in Table V-8.

The frequency of chromosomal aberrations in male Chinese hamster lung (CHL) cells incubated with styrene was similar to the frequency in controls in the absence of metabolic activation, but in the presence of the S-9

TABLE V-8

Clastogenic Effects of Styrene in Cultured Cells or Laboratory Animals

Cell Type	Type of Exposure/ End Point	Dose	Metabolic Activation	Response	Comments	Reference
CHL cells	in vitro/chromosomal aberrations	0.25 mg/mℓ	+/-	+/-	Between 10 and 19% of the cells had chromosomal aberrations in the presence of S-9, while <4.9% had aberrations in the absence of S-9.	Matsuoka et al., 1979
CHL cells	in vitro/chromosomal aberrations	NR	+/-	+/-	No specific experimental detail was provided in this report	Ishidate and Yoshikawa, 1980
human lymphocytes	in vitro/chromosomal aberrations	0.03% (v/v)	-	+	Chromosome breaks were the predominant aberrations observed.	Linnainmaa et al., 1978a,b
CHO cells	in vitro/SCE	50 1000 µg/ℓ	+/-	-/-	An increase in SCEs was observed when an epoxide hydratase inhibitor, cyclohexene oxide, was added.	de Raat, 1978
human lymphocytes	in vitro/SCE	0.5-5 mM (0.0520.52 mg/mℓ)	-	+	A dose-dependent increase in SCEs occurred starting at 0.75 mM, with an ~7-fold increase at a concentration of 5 mM.	Norppa et al., 1980a
bone marrow cells (hamster)	in vivo/chromosomal aberrations	300 ppm, 6 hours/day, 5 days/week, for either 4 days or 3 weeks (inhalation)	NA	-	The results were negative when gaps were included or excluded from the analysis.	Norppa et al., 1980b
bone marrow cells (rats)	in vivo/chromosomal aberrations	300 ppm, 6 hours/day, 5 days/week, for 3 months (inhalation)	NA	+	An increase in cytogenetic damage occurred only after 9 weeks of exposure. Interim sacrifices occurred between 2-11 weeks.	Meretoja et al., 1978a
bone marrow regenerating liver cells and alveolar macrophages (mouse)	in vivo/SCE	104, 387, 591 and 922 ppm, 6 hours/day, for 4 days (inhalation)	NA	+	SCEs were elevated in all cell types at ≥387 ppm.	Conner et al., 1979, 1980, 1982
polychromatic erythrocytes (mouse)	in vivo/micronuclei	250, 500, 1000 and 1500 mg/kg (intraperitoneal)	NA	+	A small but significant increase in micronuclei was observed at 250 and 1000 mg/kg. The high dose killed 2 of the 4 animals.	Norppa, 1981

NA = Not applicable

fraction chromosomal aberrations increased in response to styrene (Matsuoka et al., 1979; Ishidate and Yoshikawa, 1980). Matsuoka et al. (1979) reported that in the presence of S-9, 10-19% of the cells had chromosomal aberrations including chromatid gaps, breaks and exchanges, and chromosomal exchanges, ring formations and fragmentations. Styrene did not produce an increase in sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells in the presence or absence of a metabolic activation system (de Raat, 1978).

Linnainmaa et al. (1978a,b) observed a dose-related decrease in the mitotic index after _in vitro_ incubation of styrene with lymphocytes isolated from healthy human subjects. After a 72-hour incubation with styrene, the mitotic index was near control levels at a concentration of 0.008% (v/v), but decreased to 50 and 0% of control values at 0.03 and 0.08% (v/v), respectively. Chromosomal damage (primarily chromosomal breaks) was observed after a 72-hour incubation at 0.08% (v/v). The authors noted that these results may have been due to metabolically formed styrene oxide, since freshly isolated human lyomphocytes are capable of metabolic activation. Norppa et al. (1980a) also observed a dose-related increase in SCE in cultured human lymphocytes.

Preston et al. (1981) analyzed the results of _in vitro_ cytogenetic studies using cultured mammalian leukocytes in which 53 different compounds were tested. Styrene was found to be positive in inducing chromosomal aberrations in peripheral human leukocytes.

No increase in chromosomal aberrations were observed in bone marrow cells of Chinese hamsters exposed to styrene vapors at 300 pm 6 hours/day for 4 or 5 days/week for 3 weeks (Norppa et al., 1980b). Meretoja et al. (1978a) conducted a similar study, exposing male Wistar rats to 300 ppm styrene 6 hours/day, 5 days/week for 3 months. An increase in the percentage of aberrant bone marrow cells (8-12% compared with 1-6% in controls) was observed at weeks 9-12, but no change was evident earlier.

Conner et al. (1979, 1980, 1982) found inhalation exposure to styrene induced increases in SCE in mouse bone marrow, alveolar macrophages and regenerating liver cells at exposure levels $\geq$387 ppm 6 hours/day for 4 days. The duration of exposure was important since SCE in bone marrow decreased from 10.0 to 5.1 to 3.0/cell when the exposure period at the 922 ppm level was changed from 4 to 2 to 1 days, respectively. The response of bone marrow cells and alveolar macrophages did not change following partial hepatectomy (as compared with nonhepatectomized mice), which suggested to the investigators that biotransformation of styrene to styrene oxide by the liver was not a factor in the induction of SCE in extra-hepatic tissues.

Following intraperitoneal injection of male C57Bl/6 mice with dose levels of 250, 500, 1000 or 1500 mg/kg, there were statistically significant increases in micronuclei in only the 250 and 1000 mg/kg group, but the magnitude of the responses were small with only 0.99% of the cells in the 1000 mg/kg/group having micronuclei compared with 0.49% in the control (Norppa, 1981).

Styrene oxide produced a dose-related increase in SCE in the absence of S-9 in Chinese hamster ovary cells *in vitro*, while there was a decrease in the number of SCE with increasing concentrations of S-9 (de Raat, 1978). Styrene oxide also produced a dose-related increase in SCE in isolated human lymphocytes (Norppa et al., 1980a). Similar frequencies of SCE occurred with the concentration of styrene oxide 1/10 the concentration of styrene, suggesting that styrene oxide is a more potent indication of SCE than styrene (Norppa et al., 1980a). Linnainmaa et al. (1978a,b) observed an 80% decrease in the mitotic index in human lymphocytes after an 8-hour incubation with styrene oxide at 0.008% (v/v), whereas a 72-hour incubation with styrene at 0.008% (v/v) resulted in a mitotic index similar to that of the controls. No cytogenetic damage has been induced by styrene oxide following *in vivo* exposure of laboratory animals (Fabry et al., 1978; McGregor, 1981; Norppa et al., 1979).

The ability of styrene oxide to interact with DNA has also been examined. Although styrene oxide formed nonextractable products with cellular macromolecules *in vivo* (Marniemi et al., 1977), studies using cells in culture (Brouns et al., 1979) and isolated DNA (Drinkwater et al., 1978) have generally failed to demonstrated interactions of styrene oxide with DNA. A limited degree of reactivity was observed when a high concentration (0.5 M) of styrene oxide was incubated with the 4 deoxyribonucleosides of DNA (Hemminki et al., 1980b). Studies of chromosomal damage in peripheral lymphocytes of humans with occupational exposure to styrene are summarized in Chapter VI on Occupational Studies of Cytogenetic Changes.

The extent of covalent binding to plasma proteins, hemoglobin and guanine-N-7 (Gua-N-7) in DNA was determined after i.p. exposure of 7-^{14}C-styrene and 7-^{3}N-styrne-7,8-oxide (Nordquist et al., 1985). Covalent binding to plasma proteins and hemoglobin was estimated from the total radioactivity bound to the material whereas binding to DNA was estimated from the product of reaction of styrene-7,8-oxide with Gua-N-7. Gua-N-7 is presumably the site of alkylation. The metabolic incorporation of the total radioactivity into DNA was found to be ~10% for styrene and ~50% for styrene-7,8-oxide. However it should be noted that values for binding to macromolecules do not represent maximum values since the metabolism of styrene is not complete 2 hours after treatment of the animals. The degree of alkylation of plasma proteins, hemoglobin and Gua-N-7, 2 and 5 hours after treatment, are given in Table V-9. A comparison of the degree of alkylation of Gua-N-7 in DNA, plasma proteins and hemoglobin by styrene and styrene-7,8-oxide suggests that the two compounds are about equally effective as alkylating agents (Tables V-9 and V-10). Covalent binding of styrene-7,8-oxide was demonstrated in mouse DNA from liver, lung and brain (Table V-10). The DNA adduct levels (at Gua-N-7) in these tissues was comparable to that produced by ethylene oxide, a clearly-demonstrated mutagen.

Carcinogenicity. Both positive and negative bioassays are available regarding the potential carcinogenicity of styrene in experimental animals. Statistically significant increased tumor incidences were observed in some of the studies when compared with concurrent controls. In addition, these positive responses involved multiple strains, species and sexes, a dose-response effect and tumor induction at the same site by the same route in two separate studies.

TABLE V-9

Degree of Alkylation in Pooled Samples of Plasma Proteins and Hemoglobin and of Guanine-N-7 of Liver DNA in Male NMRI Mice After i.p. Injection of Styrene and Styrene-7,8-oxide, Respectively

Agent	Inj. Amount (mmol/kg bw)	Binding to Plasma Proteins[a] (nmol/g prot.)	Binding to Hb[a] (nmol/g Hb)	Binding to Gua-N-7 (nmol/g DNA)
Styrene[b]	0.12 (n-5)[c]	33 (280)		
	0.71 (n=5)	160 (220)		
	1.2 (n=5)	370 (310)		
	2.4 (n=4)	370 (150)		
	3.3 (n=5)	560 (170)		
Styrene[d]	1.1 (n=5)	200 (180)	3 (3)	17+5(15+5)
	2.3 (n=5)	260 (110)	10 (6)	25+9(11+4)
	3.3 (n=5)	290 (89)	40 (10)	
	4.9 (n=4)	430 (88)	60 (10)	31+6(6.3+1.2)
Styrene-7,8-oxide[d]	0.037 (n=2)	9.8 (260)	0.1 (3)	
	0.063 (n=9)	22 (350)	0.1 (2)	
	0.092 (n=6)	25 (270)	0.4 (4)	
	0.32 (n=6)	180 (560)	3.4 (10)	
	1.1 (n=6)	750 (680)	13 (10)	8+2(7+2)
Ethylene oxide	0.044		2 (45)	2 (45)

[a] Limit of detection; ~1 nmol/g Hb in the case of styrene and in the case of styrene oxide; ~0.004 nmol/g Hb at the lowest dose (0.037 mmol/kg bw) and ~0.1 nmol/g Hb at higher doses (0.063-1.1 mmol/kg bw)

[b] 5 hours after treatment

[c] Number of animals

[d] 2 hours after treatment

Note: Ethylene oxide, a proved mutagen, is used for comparion.

TABLE V-10

Degree of Alkylation of Guanine-N-7 in DNA of Extrahepatic Organs in the Male NMRI Mouse per mmol Styrene-7,8-oxide per kg bw 2 Hours After Treatment[a]

Organ	nmol adduct/g DNA[b]
Brain	5±2
Lung	3±1
Spleen	0.6±0.1
Testis	0.3±0.3

[a] DNA pooled from animals treated with 0.063-1.1 mmol/kg bw. Average dose estimated to 0.36 mmol/kg bw.

[b] The samples were counted 5x100 min. The 95% confidence intervals have been estimated from the radioactivity determinations.

Ponomarkov and Tomatis (1978) conducted a study to assess the carcinogenicity of styrene following oral exposure. Styrene (99% pure) in olive oil was administered once by gavage at a dose of 1350 mg/kg to 29 female mice (O_{20} strain), 300 mg/kg to 15 female C57Bl mice and 1350 mg/kg to 21 female rats (BDIV strain) on day 17 of gestation. The offspring of the C57BL mice (27 of each sex) received weekly doses (after weaning) of 300 mg styrene/kg for life. After weaning, the offspring of the O_{20} mice (45 males, 39 females) were treated weekly with 1350 mg styrene/kg, but treatment was stopped at 16 weeks because of overt toxicity. After weaning, the offspring of the BDIV rats (73 males, 71 females) received weekly doses of styrene for life at a level of 500 mg/kg. Forty-two O_{20} mice, 25 C57Bl mice and 75 BDIV rats treated with olive oil served as progeny controls. One hundred O_{20} mice and 100 C57Bl mice were also included in the study as untreated controls.

Early deaths occurred among the 45 male and 39 female styrene-treated offspring of the O_{20} mice (average age of death was 32 weeks for males and 49 weeks for females compared with 88 and 85 weeks for males and females, respectively, in the oil-treated controls). Nevertheless, a significant increase in the incidence of lung tumors classified as adenomas and carcinomas was observed for both sexes ($p<0.02$ for males and $p<0.001$ for females, calculated from study results using Fisher's exact test) when compared with the olive oil-treated controls. Characteristically, O_{20} mice have a high spontaneous rate of lung adenomas and carcinomas; in this study, the concurrent control incidence was 42%-67% (male and female) with an age at tumor onset ranging from 53-57 weeks. The lung tumor rates (based on the survivors at the time the first tumor was observed) were 20/23 and

32/32 in treated males and females, respectively, compared with 8/19 and 14/21 in male and female vehicle-treated controls, and 34/53 and 25/47 in male and female untreated controls, respectively. Lung tumors were observed in mice dying at an earlier age in the treated group as compared with the controls. The female treated mice had a much higher ratio of carcinomas to adenomas than the vehicle controls (1.3 treated compared with 0.4 in the vehicle controls) but a similar ratio to the untreated controls. The high spontaneous background rate of lung tumors in 0_{20} mice raises a question as to how to interpret this increase in tumor incidence. The observed significant increase in tumor incidence cannot be dismissed, therefore, and is thought to be of a highly suggestive nature given the high statistical significance of the response and the reduced latency period for tumor induction in terms of indicating a tumorigenic potential.

In the other animal species used in the Ponomarkov and Tomatis study, no significant styrene-related effects were reported by the authors. In C57Bl mice a slight, but insignificant, increase in liver carcinomas was observed in treated animals (3/24). Although this increase is not statistically significant when compared with male vehicle controls (0/12), it is statistically significant (p=0.022 calculated from study results using Fisher's exact test) when compared with male untreated controls (0/47) and vehicle controls combined. Pooling both control groups is proper because there is no difference between their tumor responses. No significant tumor-related effects were observed in BDIV rats. As the dosing in these assays (once per week) was not continuous, the sensitivity of these assays is thought to be less than optimum.

The National Cancer Institute (NCI) evaluated the carcinogenicity of pure styrene in groups of 50 male and 50 female Fischer 344 rats and B6C3F1 mice (NCI, 1979). Styrene was administered by gavage in corn oil 5 days/week for 78 weeks, or in the case of the low-dose rats 103 weeks. This study was terminated at 91 weeks (mice) and 104-105 weeks (rats). Increased tumor incidence was observed only in male B6C3F1 mice in this study. A dose-related increase in aveolar/bronchiolar adenomas and carcinomas was observed, with the low-dose (150 mg/kg bw) and high-dose (300 mg/kg bw) groups showing, respectively, tumor incidences of 14% (6/44) and 21% (9/43; $p=0.024$ calculated from study results using by Fisher's exact test) compared with a 0% (0/20) incidence in the vehicle control group. In spite of its positive aspects (a dose-related increase in tumor incidence with a statistically significant increase at the high dose), this study was considered by NCI to provide "suggestive" evidence for carcinogenicity in the male mouse. The validity of the lung tumor response was questioned because the concurrent vehicle control incidence of 0/20 was considered to be abnormally low, given that the historical nonvehicle control rate for this laboratory is 12% (32/271), and on two occasions has been as high as 20% (4/20). It should be noted, however, that the observed 0/20 vehicle concurrent control rate is not unprecedented inasmuch as the historical rate for vehicle controls in this laboratory was 0/40.

In a chronic bioassay limited to the investigation of chronically induced tumors of the brain, 40 male and 40 female Sprague-Dawley rats/group were treated by gavage with styrene in olive oil at levels of 0, 50 or 250 mg/kg 4-5 days/week for 52 weeks (Maltoni et al., 1982). The brain was examined for gross and histologic signs of tumors at the time of spontaneous death. The tumor incidence in treated animals was not significantly different from either the vehicle-treated controls or the historic controls. One ependymona was found in a female in the 50 mg/kg group and in the control group, 1 glioma was found in a male of the 50 mg/kg group and 1 meningioma was found in a male and female of the 250 mg/kg group and in three females of the 50 mg/kg group.

Two chronic inhalation studies have been conducted to evaluate the carcinogenicity of styrene (Jersey et al., 1978; Maltoni et al., 1982). Jersey et al. (1978) exposed groups of ~85 male and 85 female Sprague-Dawley rats to 0, 600 or 1200 ppm of styrene (99.5% pure) 6 hours/day, 5 days/week for 18-20 months. After 2 months, the concentration in the high-dose group was decreased to 1000 ppm because of decreased weight gain in the males. Exposures were terminated when 50% mortality was reached for one test group of each sex (18.3 and 20.7 months for males and females, respectively). All surviving animals were sacrificed at 24 months.

Murine pneumonia caused high mortality in males (control group and 1000 ppm group), thus making interpretations of the tumor incidence data for males difficult. In females, there was a statistically significant increase (p=0.03, Fisher's exact test) in grossly visible ovarian tumors (5/85 compared with 0/85 in control), but two of the identified tumors were not classified as such after microscopic examination. The incidence of mammary adenocarcinomas in the 600 ppm group was significantly higher (p=0.032, calculated from study results using Fisher's exact test) than in the control group (7/85 compared with 1/85 in control), but the incidence in the control group was unusually low when compared with the historical control rate for this tumor site in the same laboratory during the 5 years prior to this study.

An increased incidence of leukemia/lymphosarcomas, only in females, was also observed in the Jersey study, with both dose groups showing identical incidences of 7% (6/85) compared with a concurrent control rate of 1.2% (1/85); the historical control rate for this group of neoplasms has been as high as 2.6% in this laboratory. The absence of a dose-related increase in tumor incidence in this study at the high dose may be due to the fact that metabolic clearance of styrene is known to become saturated at levels above 200-600 ppm in the rat. The increased incidence of these neoplasms was considered by the authors of the study not to be statistically significant (p=0.06) compared with concurrent controls, although a trend was noted. With a P value of 0.06, the response would be better characterized as having a borderline significance. The increase was found to be highly significant (p=0.003 calculated from study results using Fisher's exact test), however, when compared with the historical controls for this tumor site. The study results for the males were confounded by "excessive" nontreatment-related mortality, which occurred principally in the control and high-dose groups. In view of the various uncertainties surrounding the data, this study, although giving a positive response, was considered by its authors to provide only suggestive evidence for carcinogenicity.

In an unpublished study by the Chemical Manufacturers Association, male and female Sprague-Dawley rats were administered 0, 125 and 250 ppm styrene in drinking water for 104 weeks (cited in Huff, 1984). Daily styrene intakes were estimated to be 0, 7.7 and 14.0 mg/kg for males and 0, 12 and 21 mg/kg for females. A dose-related decrease in water consumption was reported. The report concluded that under the conditions of the study, styrene in drinking water at 0, 125 and 250 ppm did not produce deleterious effects in the rat. According to the data a marginal increase in mammary gland neoplasms were observed in female rats. The combined incidence of fibroadenoma, adenoma and adenocarcinoma in the 250 ppm dose group was significantly higher ($p=0.039$) than the controls. However the increase was deemed marginal (cited in Huff, 1984).

Maltoni et al. (1982) determined the incidence of brain tumors following inhalation exposure of rats to styrene. Groups of 40 male and 40 female Sprague-Dawley rats were exposed to 0, 25, 50, 100, 200 or 300 ppm styrene 4 hours/day, 5 days/week for 52 weeks. After spontaneous death, the brain was examined for gross and microscopic lesions. The only tumors detected were one glioma in a female exposed to 25 ppm and one meningioma in a male from both the 25 and 100 ppm groups and in three females of the 100 ppm group. Styrene was not considered to induce brain tumors.

A long-term carcinogenicity bioassay by Conti et al. (1988) tested styrene by inhalation 4 hours/day, 5 days/week for 52 weeks in Sprague-Dawley rats at 0, 25, 50, 100, 200 and 300 ppm (30 animals/sex/dose). Styrene and styrene oxide were tested by ingestion (stomach tube) in olive oil once daily, 4-5 days/week for 52 weeks at 0, 50 and 250 mg/kg (40 rats/sex/dose). Styrene was also tested in olive oil by intraperitoneal injection (4 times) at 0 and 50 mg and subcutaneous injection (una tantum) at 0 and 50 mg (40 rats/sex/dose). Animals were kept under observation until spontaneous death. The rats were clinically examined for gross changes every 2 weeks, weighed every 2 weeks during treatment and then every 8 weeks. Full necropsy was performed on all animals. A higher mortality rate was observed in female rats treated by ingestion with the high dose of styrene. There was a higher incidence of total malignant tumors in the group exposed to styrene at 100 ppm by inhalation and a higher incidence of total (benign and malignant) mammary tumors and malignant mammary tumors in the females of all the groups exposed to styrene by inhalation when compared with controls. The incidence of malignant mammary tumors was considered to be treatment related and statistically significant. There was an increased incidence in mammary tumors (benign and malignant) in the low-dose females following ingestion. However, as a consequence of the higher mortality rate, a lower incidence of total benign and malignant tumors and of total mammary tumors was observed in rats treated by styrene ingestion at the highest dose level. Styrene oxide-treated males exhibited a slight increase in mortality rate for both treated groups. A dose-related increase in total and malignant tumors was observed in styrene oxide-treated groups. This increase is due to an increase in forestomach neoplasias.

Preliminary results of a chronic bioassay of styrene oxide (a styrene metabolite) have been reported by Maltoni et al. (1979). Styrene oxide was administered by gavage to groups of 40 male and 40 female Sprague-Dawley rats at doses of 0, 4.12 or 8.25 mg/kg 4-5 times/week for 52 weeks. The authors dosing schedule was not clear. Complete autopsies were performed at death. This preliminary report described the incidence of stomach tumors diagnosed up to the 135th week of the study (duration of the study was 156 weeks). The liver was reported to be a common site of metastasis and multiple tumors were often observed. There were preneoplastic lesions in the forestomach in all animals. Maltoni et al. (1982) reported that there was no significant increase in the incidence of brain tumors in this bioassay. The only tumors observed were meningiomas found in one high- and one low-dose female, one low-dose male and one male control. The histology results from the other tissues examined have not been reported. Styrene oxide produced a dose-related increase in the incidence of stomach tumors and no change in the incidence of brain tumors in this study.

Styrene oxide has also been assayed for carcinogenicity administered by gavage to F344 rats and B6C3F1 mice for 2 years (Lijinsky, 1986). Styrene oxide (96.6% purity) was administered in corn oil 3 times/week at two dose levels to groups of 52 animals of each sex. The high and low doses per treatment given to rats were 550 and 275 mg/kg bw; the doses given to mice were 750 and 375 mg/kg bw. Control animals (52 of each sex and species) were given corn oil only. The doses employed did not appear to subject the animals to excessive toxic stress. All styrene oxide treatment groups exhibited high incidences of forestomach carcinomas plus papillomas. In rats the incidences ranged from 46/52 (low-dose females) to 50/52 (high-dose males). In mice, the incidence range was 24/50 (high-dose females) to 37/51 (low-dose males). Only 1/104 control rats and 2/104 control mice had this lesion. Furthermore, the low-dose male mice showed a significantly higher ($p<0.001$) incidence of hepatocellular adenomas plus carcinomas (28/52) than controls (12/51). It was concluded that styrene oxide was a carcinogen under the conditions of this assay. The authors also suggested that rats were more susceptible to styrene oxide than mice since rats tended to have a higher incidence of forestomach neoplasms at a lower dose.

Styrene oxide also produced benign and malignant forestomach tumors in male and female BDIV rats (Ponomarkov et al., 1984). Fourteen female rats were administered a single oral dose of 200 mg/kg bw styrene oxide (in corn oil) by gavage on day 17 of gestation. The progeny (43 males and 62 females) were administered 96 weekly doses of 100-150 mg/kg bw styrene oxide from 4 weeks of age until termination of the experiment at 120 weeks. Control animals (14 pregnant females, 55 female and 49 male progeny) were given corn oil only. Complete autopsies were conducted along with routine histopathologies. The number of tumor bearing animals was 46/60 (77%) and 22/42 (52%) in female and male progeny, respectively. The incidence of forestomach papilloma in female progeny was not significant (2/60) however, male progeny incidence were 7/42 ($p<0.003$); forestomach carcinomas were significant in both sexes. The incidences were 6/60 ($p<0.02$) in females and 4/42 ($p<0.04$) in males. The control rats incidence of this lesion was 0/55 (females) and 0/49 (males). It is important to note in addition to forestomach tumors two other types of tumor rarely found in BDIV rats were noted. Four tumors of the nervous system occurred in treated rats with only one observed in a male control. Seven lung tumors occurred in treated progeny with only two in controls. Six of the lung tumors occurred in females compared with one in control females. The incidence of these tumor types (lung and nervous system) did not reach levels of statistical significance. Ponomarkov et al. (1984) concluded that styrene oxide is a direct-acting carcinogen producing tumors of the forestomach.

The results from two studies in which styrene oxide was tested by skin painting did not demonstrate a carcinogenic potential for styrene oxide in this assay system. No skin tumors were observed among 40 C3H mice painted with a 5% solution of styrene oxide in acetone 3 times/week for life (beginning at 12 weeks of age) (Weil et al., 1963). Similar application of a 10% styrene oxide solution resulted in high mortality (only 2/40 survived until the 17th month). Van Duuren et al. (1963) painted 30 male Swiss ICR/Ha mice with 0.1 mℓ of a 10% styrene oxide solution in benzene twice weekly for life (from 8 weeks old). The median lifespan of the test animals was 431 days compared with ~300 days for the 150 benzene tested controls. A total of three tumors (10%) developed in the 30 treated mice including one malignant tumor, while in the controls, 11 tumors (7%) developed, including one malignant tumor at a site remote from the application.

In another skin painting study briefly reported by Kotin and Falk (1963), a 16% (3/20) incidence of malignant lymphomas was observed in C3H mice painted with styrene oxide. The total dose was reported to be 20 μM. Since this is a concentration term, and the volume applied was not stated, the actual dose given is not clear. No skin or lung tumors were reported. No experimental details were provided.

Summary

The acute toxicity of styrene is relatively low. Wolf et al. (1956) reported an acute oral LD_{50} of ~5000 mg/kg for rats treated by gavage. The highest single oral dose resulting in no mortality was 1600 mg/kg, while the lowest dose resulting in 100% mortality was 8000 mg/kg (Spencer et al., 1942). No mortality resulted in rabbits following dermal applications of styrene of ≤20,000 mg/kg for 24 hours (Harton and Rawl, 1976). LC_{50} values ranging from 2700 ppm for 4 hours (Jaeger et al., 1974) to 4620 ppm for 6 hours (Bonnet et al., 1982) have been reported from rat inhalation studies. Reported LC_{50} values for mice were 4930 ppm for 4 hours

(Shugaev, 1969) and 2430 ppm for 6 hours (Bonnet et al., 1982). The acute toxicity of styrene oxide, a styrene metabolite, is higher than for styrene. Weil et al. (1963) reported an oral LD_{50} in rats of 2550 mg styrene oxide/kg.

Spencer et al. (1942) found no apparent toxic effects of oral exposure of rats to styrene at doses of 100 mg/kg, 5 days/week for 4 weeks, but reduced weight gains were observed at 500 mg/kg and a few deaths were reported at $\geq$1000 mg/kg. No histopathologic or hematologic effects were noted in rats at oral doses $\leq$667 mg/kg for 6 months (5 days/week); kidney, liver and body weight changes were observed at $\geq$400 mg/kg (but not at <133 mg/kg) (Wolf et al., 1956). Severe toxic effects (mortality, liver necrosis, severe lung congestion) were observed in mice after weekly oral doses of 1350 mg/kg for <16 weeks (Ponomarkov and Tomatis, 1978). Beliles et al. (1985) found no toxic effects to rats when exposed to 7.7 and 14 and 12 and 21 mg/kg/day styrene in the drinking water for 2 years. Forestomach and kidney lesions were reportedly common (incidences not given) in a chronic study of rats dosed orally with 500 mg/kg/week (Ponomarkov and Tomatis, 1978).

Changes in enzyme activity following oral exposure to styrene have been studied by a number of investigators. An increase in mixed function oxidase activity has been reported in rat liver after seven oral doses of 450 or 900 mg/kg/day, but not 270 mg/kg/day (Das et al., 1981a) and after oral doses of 200 or 400 mg/kg 6 days/week for 100 days (Srivastava et al., 1982). A dose-related decrease in hepatic glutathione-S-transferase activity was observed after oral dosing with 200 or 400 mg/kg 6 days/week for 100 days

(Srivastava et al., 1982), while both hepatic and renal glutathione-S-transferase activity decreased significantly after 450 or 900 mg/kg (but not 270 mg/kg) oral doses for 7 days (Das et al., 1981a).

Minor hepatotoxic effects of oral exposure to styrene have been indicated. Srivastava et al. (1982) found levels of SGOT and SGPT were elevated and areas of focal necrosis were present in rat liver at a dose level of 400 mg/kg 6 days/week for 100 days. Quast et al. (1978, 1979) found minimal histopathological changes in dogs following subchronic oral exposure to styrene (600 mg/kg for 560 days). In a chronic study, small necrotic foci were observed in the liver of rats dying at ~1 year of age; these changes were not observed in rats dying later (Ponomarkov and Tomatis, 1978).

In the brain, oral administration of ~900 mg/kg for 15 days resulted in significantly elevated levels of noradrenalin and serotonin and depressed activity of monoamine oxidase in rats (Husain et al., 1980), while increased sensitivity of dopamine receptors was observed at oral doses of 200 and 400 mg/kg for 90 days (Agrawal et al., 1982).

Results of inhalation studies of styrene support the oral exposure results. Alterations in hepatic enzyme activity were observed during the 11-week exposure of rats to 300 ppm styrene (6 hours/day, 5 days/week) (Vainio et al., 1979) and after 7 days of exposure to 450 ppm 8 hours/day (Sandell et al., 1978a,b). Minor histological liver alterations were found after <2 weeks of exposure to 300 ppm, 6 hours/day, 5 days/week (Vainio et al., 1979). Altered enzyme activities were observed in the brain near the end of 11-week exposure to 300 ppm x 6 hours/day (5 days/week) (Savolainen and Pfaffli, 1977). Glial cells were apparently not affected by similar exposure (Savolainen et al., 1980). A transient increase in the motor conduction velocity of the tail nerve of rats was observed at exposures to 300 pm 6 hours/day, 5 days/week (Seppalainen, 1978).

Weight depression was observed in rats at exposures of 2000 ppm styrene (7 hours/day for 5 days/week for 6 months), but not at 1300 ppm (Spencer et al., 1942; Wolf et al., 1956). In guinea pigs, no adverse effects were observed at 650 ppm (exposure schedule same as for rats); 10% mortality was observed after a few exposures to 1300 ppm, but mortality was apparently not affected at 2000 ppm. De Ceaurriz et al. (1983) noted a behavioral change (increased swimming activity) in mice following a single 4-hour inhalation exposure to styrene (800-850 ppm). In a chronic inhalation study with female rats exposed to 600 or 1000 ppm 6 hours/day 5 days/week, no gross or histopathological changes were considered exposure-related except for several minor lung effects and liver and body weight changes (Jersey et al., 1978).

The substrate affinity of several hepatic enzymes was altered following intraperitoneal doses of styrene (Lambotte-Vandepaer et al., 1979). Glutathione depletion was observed in the liver following acute styrene exposure of laboratory animals (Vainio and Makinen, 1977; Das et al., 1981a; Parkii et al., 1978). Glutathione depletion also occurred following oral dosing of rats with 900 mg/kg (but not 450 mg/kg) for 7 days (Das et al., 1981a), and following subchronic inhalation exposure (Vainio et al., 1979).

No teratogenic effects of styrene have been observed in rats exposed orally (Murray et al., 1976, 1978a; Beliles et al., 1985) or in rats, mice, rabbits and hamsters following inhalation exposure (Murray et al., 1978a,b; Ragule, 1974; Kankaanpaa et al., 1980). The doses or exposure levels were sufficiently high to cause some maternotoxic effects in the oral and inhalation studies conducted by Murray et al. (1976, 1978a,b). Fetotoxicity (increased incidence of resorptions or dead fetuses) was reported by Kankaanpaa et al. (1980) in mice exposed to 250 ppm 6 hours/day, on days 6-16 of gestation and in hamsters exposed to 1000 ppm 6 hours/day, on days 6-18 of gestation (but not in hamsters exposed to <750 ppm).

In mutagenicity assays, there was no increase in the number of revertant colonies of *Salmonella typhimurium* when styrene was assayed in the absence of a mammalian metabolic activating system (De Meester et al., 1977, 1981; Busk, 1979; De Flora, 1981; Loprieno, 1977; Milvy and Garro, 1976; Poncelet et al., 1980; Simmon et al., 1977; Stoltz and Withey, 1977; Vainio et al., 1976), but in the presence of a metabolic activating system, there were mixed positive and negative results (Stoltz and Withey, 1977; De Flora, 1981; Busk, 1979; Loprieno et al., 1978; Simmon et al., 1977; De Meester et al., 1977, 1981; Vainio et al., 1976; Poncelet et al., 1980). Styrene oxide was mutagenic in *S. typhimurium* with and without metabolic activation (De Meester et al., 1977, 1981; Busk, 1979; De Flora, 1981; Glatt et al., 1975; Loprieno et al., 1978; Milvy and Garro, 1976; Vainio et al., 1976; Drinkwater et al., 1978). Watabe et al. (1982b) found that another presumed styrene metabolite, 1-vinylbenzene-3,4-oxide, was also mutagenic. The results of mutagenicity assays of styrene in eukaryotic organisms were largely negative (Loprieno et al., 1976; Bauer et al., 1980; Beije and

Jenssen, 1982), but positive responses were observed in a host-mediated assay with _Saccharomyces cerevisiae_ (Loprieno et al., 1976), in mammalian cells (V-79) _in vitro_ with metabolic activation provided by a liver perfusion system (Beije and Jenssen, 1982), and in a recessive lethal assay using _Drosophila melanogaster_ (Donner et al., 1979)

Increased incidences of chromosomal damage as a result of styrene exposure have been reported in animal cells _in vitro_ with (but not without) metabolic activation (Matsuoka et al., 1979; Ishidate and Yoshikawa, 1980), in isolated human lymphocytes (Linnainmaa et al., 1978a,b; Norppa et al., 1980a) and in rat and mouse cells (bone marrow or other) _in vivo_ following inhalation exposure or intraperitoneal injection (Meretoja et al., 1978a; Conner et al., 1979, 1980, 1982; Norppa, 1981). Recent evidence showed that styrene and its epoxide form DNA adducts (Nordqvist et al., 1985).

Several carcinogenicity studies have reported statistically significant increases in the incidences of tumors at specific sites in specific test groups following styrene exposure. There were increased tumor incidences observed in four studies (NCI, 1979; Jersey et al., 1978; Ponomarkov and Tomatis, 1978; Conti et al., 1988). Some increases in tumor incidences were statistically significant when compared with concurrent controls. Direct evidence that inhalation of styrene causes cancer in laboratory animals comes from the study by Jersey et al. (1978) and Conti et al. (1988). Groups of male or female Sprague-Dawley rats (Jersey et al., 1978) were exposed for 6 hours/day, 5 days/week to styrene vapor at 600 or 1200 ppm. The high dose was reduced to 1000 ppm after 2 months. The study was terminated after 24 months. A statistically significant increases in mammary adenocarcinoma was observed in females exposed to 600 ppm. Conti et al. (1988) exposed male and female rats 4 hours/day, 5 days/week for 52 weeks to styrene at 0, 25, 50, 100, 200 and 300 ppm by inhalation. There was a higher incidence of total (benign and malignant) mammary tumors and malignant mammary tumors in the females of all groups exposed to styrene when compared with controls.

Evidence that ingestion of styrene causes cancer in animals comes from two studies. Ponomarkov and Tomatis (1978) administered styrene by olive oil gavage to female O_{20} mice, C57Bl mice, and BDIV rats once on the 17th day of gestation and then weekly throughout their offspring's lifetimes. A statistically significant increased incidence and earlier onset of lung tumors were observed in the O_{20} offspring, but there was a high background tumor rate in this strain. A few rare tumors were observed in the BDIV offspring. In the C57Bl offspring there was a statistically significant increased incidence of liver tumors compared with pooled, untreated and vehicle controls.

NCI (1979) administered styrene at 150 or 300 mg/kg daily by corn oil gavage to B6C3F1 mice. Exposure was terminated after 78 weeks, and the study was terminated after 91 weeks. Statistically significant increased incidences of lung alveolar/bronchiolar adenomas or carcinomas were observed in both exposed groups with a statistically significant dose-related trend.

Negative results were observed in a chronic bioassay investigating induced tumors of the brain (Maltoni et al., 1982). Sprague-Dawley rats were exposed to styrene in olive oil by gavage (0, 50 or 250 mg/kg) or inhalation to styrene vapors (0, 25, 50, 100, 200 or 300 ppm). Styrene was not considered to induce brain tumors.

Styrene oxide, a metabolite of styrene, produced a dose-related increase in the incidence of stomach tumors in rats (oral doses of 4.12 or 8.25 mg/kg 4-5 times/week for 52 weeks) (Maltoni et al., 1979). Styrene oxide administered by gavage 3 times/week for 2 years to male and female F344 rats and B6C3F1 mice (low and high doses of 275 and 550 mg/kg bw for rats and 375 and 750 mg/kg bw for mice) produced high incidences of forestomach carcinomas plus papillomas in all treatment groups. Further, the incidence of hepatocellular adenomas plus carcinomas was significantly elevated in the low-dose male mice relative to controls (Lijinsky, 1986). Styrene oxide (100-150 mg/kg bw) produced statistically significant benign and malignant forestomach tumors in both sexes of BDIV rats after gavage administration for 96 weeks. In addition two other tumor types (lung and nervous system) rare to this strain were noted; however their incidences did not reach statistically significant levels (Ponomarkov et al., 1984). Styrene oxide (50 and 250 mg/kg) produced dose-related increases in total and malignant tumors in Sprague-Dawley rats when treated by ingestion (stomach tube) once daily, 4-5 days/week for 52 weeks.

When the animal bioassay data and the metabolic/genotoxicity data are considered, there is a reasonable basis for classifying styrene as having a "sufficient" level of carcinogenic evidence in animal test systems. The classification of sufficient animal evidence comes from the combined animal bioassays showing statistically significant increased tumor incidences in the B6C3F1 male mouse (alveolar/bronchiolar adenomas and carcinomas) (NCI, 1979), in male and female O_{20} mice (lung adenomas and carcinomas), in male C57Bl mice (liver carcinomas) (Ponomarkov and Tomatis, 1978) and in female Sprague-Dawley rat (mammary adenocarcinoma) (Jersey et al., 1978).

All available information points to the importance of kinetic modeling in determining the effective dose in the animal studies and in projecting the results to low-dose human exposures. These analyses are currently in progress.

VI. HEALTH EFFECTS IN HUMANS

Experimental Studies

Controlled experiments involving inhalation exposure of human volunteers have been conducted to identify irritation levels, neurological symptoms and other subjective effects. Drowsiness, listlessness and an altered sense of balance was reported during a 4-hour exposure of two male subjects to 3407 mg styrene/m^3 (800 ppm) (Carpenter et al., 1944). Eye and throat irritation with increased nasal secretion occurred immediately after exposure began, while muscular weakness was reported after exposure was terminated. Steadiness was measured by the subject's ability to hold a 1/16 inch wire in a 1/4-inch hole without touching the walls of the hole. The length of time the wire touched the walls was more than 6 times greater after exposure than before exposure.

Nine healthy male human volunteers were exposed to styrene vapor concentrations of ~50-376 ppm for periods of 1-7 hours (Stewart et al., 1968). Nine additional volunteers served as controls. Urinalysis, hematology and blood chemistry studies, which were conducted before the exposures, were repeated 16 and 72 hours following the exposures. The results of these clinical studies were normal and unchanged from pre-exposure values. Volunteers exposed to 50 ppm (217 mg/m^3) and 117 ppm (499 mg/m^3) for 1 and 2 hours, respectively, showed no signs of toxicity. The moderately strong initial styrene odor diminished after 5 minutes. At 216 ppm (921 mg/m^3), nasal irritation resulted after 20 minutes. Eye and nose irritation, strong odor and altered neurological function were reported for volunteers exposed to 376 ppm (1602 mg/m^3) for 1 hour. Most volunteers exposed to 376 ppm exhibited reduced performance in the Crawford Manual Dexterity Collan and Pin Test, the modified Romberg test, and the Flannagan Coordination test. In addition, some of these subjects reported feelings of nausea or inebriation. Six subjects were exposed to 99 ppm (422 mg/m³) styrene vapor for two 3.5-hour periods separated by a 30-minute period without exposure. There were no objective signs of impaired balance or coordination during the 7 hours. The scores of the Flannagan Coordination tests and the Crawford Manual Dexterity tests were unchanged from pre-exposure scores. Three of the six subjects did report intermittent difficulty performing the modified Romberg test. Clinical laboratory studies following exposure were normal and unchanged from the pre-exposure values.

Gamberale and Hultengren (1974) exposed 12 subjects to styrene concentrations of 213, 639, 1065 and 1491 mg/m³ (50, 150, 250 and 350 ppm) during four consecutive 30-minute intervals. Manual dexterity and perceptual speed were not affected significantly by styrene exposure, but a dose-related increase in single reaction time was evident. Reaction time recorded during the final 30-minute exposure was significantly ($p<0.05$) increased. Increased salivation, decreased (not significant) heart rate and a strong metallic-like taste also resulted from exposure.

Odkvist et al. (1982) studied the effects of styrene on the vestibulo-oculomotor functions in 10 healthy subjects exposed to 370-591 mg/m³ 87-139 ~~(88-140~~ ppm) for ∼80 minutes. Rotatory and optokinetic nystagmus were not affected, but the rate of movement of the eyes between two alternating light sources (saccade) increased significantly ($p<0.05$) after exposure. Suppression of the vestibulo-oculomotor reflex was also affected.

The reported level at which styrene odor was not detectable was <42.6 mg/m³ (10 ppm) (Wolf et al., 1956). Styrene concentrations between 426-1504 mg/m³ (100-350 ppm) resulted in strong odor, while at ≥2556 mg/m³ (600 ppm) intense eye and nose irritations resulted (Wolf et al., 1956). Alexander et al. (1982) reported an average odor threshold for styrene of 3.6 μg/ℓ in 60°C water and the average taste threshold was 0.12 mg/ℓ in 40°C water.

Epidemiological Studies

Subjective Effects. The effects of occupational exposure to styrene vapors in air have been studied by numerous investigators. The symptoms most commonly reported in styrene-exposed workers are mucosal irritation (eyes and nasopharynx), drowsiness and fatigue and difficulties in concentration (Wilson, 1944; Rogers, 1955; Zielhuis, 1961; Zielhuis et al., 1964; Gotell et al., 1972; Lorimer et al., 1976, 1978; Harkonen, 1977; Axelson and Gustavson, 1978; Rosen et al., 1978; Lilis et al., 1978; Cherry et al., 1980). These effects are typical of all aromatic hydrocarbons, are reversible upon removal from exposure and appear to be related more to exposure intensity than duration.

Subjective effects of styrene exposure were reported by Lorimer et al. (1976, 1978) from the results of a comprehensive survey of 488 styrene production/polymerization plant workers with exposure durations ≤32 years. About 98% of the workers were male and the median age was in the 40's. A high exposure group of 182 workers with estimated TWA exposure of ~5-20 ppm (with unquantitated wide excursions) was compared with a low exposure group (estimated TWA exposures of <1 ppm; 306 workers); an unexposed control was not used. Significant benzene exposure was possible in certain job categories. Acute prenarcotic symptoms occurred in a significantly ($p<0.01$) higher percentage of the high exposure group (34/182, 19%) compared with the low group (30/306, 10%).

Harkonen (1977) administered questionnaires to 98 male laminating workers (median age = 28) from 24 polyester manufacturing plants with exposure durations ranging from 0.5-14 years. An unexposed age-matched group of 98 male postal and electrical workers served as a control group. Exposure was measured by urinary mandelic acid concentration; the median concentration of the exposed group was 808 mg/ℓ. General symptoms of mucous membrane irritation, mental concentration difficulties and fatigue were reported significantly ($p<0.01$) more often in the exposed group. Prenarcotic symptoms (dizziness, nausea, drunken feeling) during the workday were also reported significantly ($p<0.001$) more often in the exposed group. None of the symptoms were significantly correlated with the concentration of urinary mandelic acid.

Groups of 13, 10 and 10 workers exposed to TWA concentrations of 125, 47 and ≤ 5 ppm styrene, respectively, and 6 unexposed workers (controls) were studied by Rosen et al. (1978). Details of the study groups are given in Table VI-1. Exposure-related effects included unusual tiredness (reported in 85, 40, 20 and 17% of the workers in the 125, 47, ≤ 5 ppm and control groups, respectively), reduction in short-term memory (62, 40, 30 and 17%) and giddiness (54, 40, 10 and 0%).

TABLE VI-1

Behavioral and Neurophysiological Effects of Occupational Styrene Exposure (Inhalation)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
17 Swedish male reinforced plastic workers; median age = 28	17 age-matched subjects from motor workshop	few days to ~12 years	>150 ppm (high) <150 ppm (low) TWA	The 6 subjects in the high exposure groups had significantly ($p<0.01$) longer mean simple reaction times when measured both before and after work compared with their controls. No difference in reflexes, vibration sensitivity or Romberg test in either group.	Gotell et al., 1972
106 Swedish boat manufacturers	NC	0.1-11 years mean = 2.7	13-101 ppm mean concentration	Longer and more irregular reaction times in exposed workers; effects were still evident in the morning (after a nights rest). Further details of results not available.	Gamberale et al., 1975
7 Swedish boat manufacturers	NC	mean 10 ± 7 years	9±4 ppm^a mean concentration	Exposed workers had longer reaction times in afternoon than in morning, whereas afternoon reaction times were shorter in controls. No effect on tests (unspecified) of sensory and motor functions.	Kjellberg et al., 1979
27 British fiberglass boat hull manufacturers; mean age = 23	27 unexposed workers from same factory; mean age = 26	NR	11-191 ppm TWA mean = 92 ppm	Five objective tests used (simple reaction time, vigilance task, digit-symbol substitution, forward and backward digit-span tests). The only significant ($p<0.05$) effect was a slower mean reaction time in exposed workers in the morning after a weekend of no occupational exposure. At the end of the work shift reaction time was similar to controls.	Cherry et al., 1980

TABLE VI-1 (cont.)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
81 West German styrene-exposed workers	34 unexposed workers	NR	NR	Significant effects of exposure intensity noted on reasoning, word fluency, substitution, cancellation, perceptual accuracy, interference, digit reproduction and choice reaction. Significant effects of exposure duration noted on digit reproduction, interference, perceptual accuracy and cancellation tests. Further details not reported.	Seeber et al., 1979
98 Finnish male laminating workers, mean age = 30	43 male concrete reinforcement mean age = 33	0.5-14 years mean = 4.9	7-674 mg/ℓ^b (low) or 1762-4715 mg/ℓ (high)	10 tests measuring general intelligence, visuomotor speed, visuomotor accuracy, memory, vigilance, psychomotor performance and personality performed. Only 2 variables differed significantly ($p<0.05$) between exposed and control: poorer visuomotor accuracy and longer latency time in Rorschach test in exposed workers. No significant difference in reaction time. Workers with high levels of urinary mandelic acid had significantly ($p<0.05$) poorer visuomotor accuracy and psychomotor performance than low exposure group.	Lindstrom et al., 1976
Further analysis of Lindstrom et al. (1976) study				Change in visuomotor accuracy first noticeable when urinary mandelin acid concentration >800 mg/ℓ (~36 ppm 8-hour estimated TWA). More pronounced decrement in visuomotor and psychomotor performance at an estimated 8-hour TWA of >55 ppm (mean mandelic acid concentration >1200 mg/ℓ).	Harkonen et al., 1978
Further analysis of Lindstrom et al. (1976) study				Personality reactions evaluated using 20 Rorschach variables. Fewer emotional reactions, lower anxiety and a lower number of neurotic signs in exposed group.	Lindstrom and Martelin, 1980

TABLE VI-1 (cont.)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
96 of 98 Finnish workers described in Lindstrom et al. (1976) study	None	0.5-14 years mean - 5	7-4715 mg/ℓ^b	23/96 (24%) of workers had abnormal EEGs; significantly ($p<0.01$) higher than expected for general population (10%). Subjects with abnormal EEGs had significantly ($p<0.05$) higher mandelic acid concentrations than subjects with normal EEGs. Proportion of abnormal EEGs was normal among workers with mandelic acid concentrations <700 mg/ℓ (4/38, 10%), but was higher among workers with higher styrene exposure (19/58, 33%). Exposure duration did not affect proportion of abnormal EEGs, but longer-exposed workers had lower mandelic acid concentrations.	Seppalainen and Harkonen, 1976
Further analysis of EEG study above				A marked change in proportion of normal and abnormal EEGs when mandelic acid concentrations >700 mg/ℓ (31 ppm estimated 8-hour TWA). Proportion of abnormal EEGs reached a plateau at 800 mg/ℓ.	Harkonen et al., 1978
40 of 96 Finnish workers described above; mean age - 30	30 males mean age - 30	NR	30-3811 mg/ℓ^b	No significant differences found in nerve conduction velocity tests (maximal motor conduction velocity of median, ulnar, deep peroneal and posterior tibial nerves, conduction velocity of slower motor fibers of ulnar and deep peroneal nerves, sensory conduction velocity of median and ulnar nerves).	Seppalainen and Harkonen, 1976

TABLE VI-1 (cont.)

Study Population	Control Population	Exposure Duration	Exposure Level	Effect	Reference
13 male polyester resin workers; mean age - 44	6 male hospital transportation service workers mean age - 36	1-12 years	125 ppm TWA (range 74-175 ppm)	Incidence of several subjective symptoms (tiredness, reduced short-term memory and giddiness) exposure-related based on questionnaire results. Nerve conduction measurements: mean duration of sensory action potentials (SAPs) at the wrist significantly ($p<0.05$) longer for the 125 and 5 ppm groups compared with controls. Amplitude of the SAPs significantly ($p<0.05$) lower in the 47 ppm group. No significant difference between groups in motor conduction velocity (of median, fibular and posterior tibial nerves) or distal motor latency of median nerve. Further comparisons and EEG analysis discussed in text.	Rosen et al., 1978
10 male polyester resin production workers; mean age - 43		2-14 years	47 ppm TWA (range 21-67 ppm)		
10 male polystyrene production workers; mean age - 44		5-15 years	<5 ppm TWA^c		
18 plastic industry workers	None	mean 1.9 years	43-130 ppm	13 (72%) workers had abnormal EEG findings.	Klimkova-Deutschova, 1962
14 female styrene-exposed workers	None	NR	NR	9 (64%) workers had abnormal EEG findings; occurrence of theta waves was the most frequent abnormality.	Roth and Klimkova-Deutschova, 1963
412 styrene production/polymerization plant workers	None	<32 years	<1 ppm or ~5-20 ppm TWA	Distal hypoethesis (touch and pain) observed in 24 cases (6%), hyperactive deep tendon reflexes observed in 15 cases (4%) and decreased deep tendon reflexes observed in 13 cases (3%). Radial nerve conduction velocities slow (<55 m/sec) in 15/80 cases; peroneal nerve conduction velocities slow (<40 m/sec) in 12/73 cases. No comparison with control or general population.	Lilis et al., 1978

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[a] Concurrent acetone exposure (35 ± 19 ppm mean concentration)

[b] Measured as mean urinary mandelic acid concentration

[c] Concurrent isopentane exposure in some workers ("minor" significance)

NC - Not characterized; NR - not reported

__Behavioral and Neurophysiological Effects__. Studies discussing nervous system effects of styrene exposure are summarized in Table VI-1. Increased reaction times of workers occupationally exposed to styrene have been reported by several investigators (see Table VI-1). Gotell et al. (1972) found significantly increased reaction times in the morning and afternoon in a group of 6 male fiberglass reinforced polyester plastic product workers exposed to >150 ppm styrene as compared with unexposed controls. Reaction times did not differ significantly in the group exposed to <150 ppm. Reaction times were longer and more irregular in 106 exposed workers (13-101 ppm styrene) compared with a control group (Gamberale et al., 1975). Workers exposed to styrene (9 ppm) and acetone had longer reaction times in the afternoon than in the morning, whereas reaction times decreased in the afternoon among unexposed controls (Kjellberg et al., 1979).

Cherry et al. (1980) found no significant differences in performance on four behavioral tests between exposed (11-191 ppm TWA) and unexposed workers. The control group worked in the same factory and had a similar education and mean age. Simple reaction time was significantly longer on Monday morning in exposed workers, but mean reaction times in the afternoons were not significantly different. There was a significant ($p<0.001$) correlation between mandelic acid concentration and start-of-shift reaction time in a subgroup of 14 men who had given morning urine samples (Cherry et al., 1981). In a follow-up study 21 months later when exposure levels were lower, the three men with the highest original mandelic acid concentrations had significantly ($p<0.05$) decreased reaction times and decreased mean concentrations of mandelic acid; reaction times were unchanged in five other workers retested (Cherry et al., 1981). Cherry et al. (1981) reported the results of a study of 17 male fiberglass workers (mean age 40 years) exposed to styrene-based resin (mean exposure 20 ppm). There was a significant ($p<0.01$) correlation between mandelic acid concentration and reaction time, but the correlation was due mainly to high mandelic acid concentration of two men with "appreciably" slower reaction times.

As summarized in Table VI-1, Seeber et al. (1979) reported performance in a number of psychological tests was correlated with intensity or duration of exposure to styrene (after adjustments for age). Lindstrom et al. (1976) studied psychological functions of 98 male laminating workers from 24 polyester manufacturers as part of an extensive cross-sectional study. The control group of 43 male workers had similar mean age, education and geographic location. Mean urinary mandelic acid concentrations was determined from five weekly measurements. The psychological performance of the styrene exposure group was similar to the unexposed group; only two of the 30 variables tested differed significantly ($p<0.05$). Workers with high styrene exposure (>1762 mg/ℓ mandelic acid) had significantly poorer visuomotor accuracy and psychomotor performance than workers with lower exposure (<674 mg/ℓ mandelic acid). The relationship between exposure duration and psychological functions was examined by stepwire regression analysis. One visuomotor speed and one memory variable significantly ($p<0.05$) increased the covariance between exposure duration and the predictor variables; their joint partial correlation with exposure duration was significant ($p<0.05$), but low (~0.28). The function with the strongest relationship to mandelic acid concentration was visuomotor inaccuracy, while psychomotor performance and vigilance were slightly related. Other statistical analyses also indicated a relationship between high mandelic acid concentrations and visuomotor inaccuracy.

These results were analyzed further to define a threshold exposure for deterioration in psychological functions (Harkonen et al., 1978). Mean urinary mandelic acid levels were converted to 8-hour TWA concentrations using the results of Engstrom et al. (1976). Discernible changes in visuomotor accuracy occurred when the urinary mandelic acid concentration was >800 mg/ℓ(~36 ppm 8-hour estimated TWA). Both visuomotor and psychomotor performance exhibited a more pronounced decrement when the mean mandelic acid concentration was >1200 mg/ℓ (~55 ppm 8-hour TWA).

Lindstrom et al. (1978) also interviewed the 98 workers about their alcohol consumption habits. Alcohol consumption, changes in alcohol consumption and lowered tolerance were not significantly related to duration or degree of styrene exposure (measured by mean mandelic acid concentrations). The author concluded from their analyses that the psychological symptoms related to styrene exposure were not the same as those relating to alcohol consumption.

Personality reactions of the same group of 98 workers and controls were evaluated by means of 20 Rorschach variables (Lindstrom and Martelin, 1980). The styrene-exposed group exhibited fewer emotional reactions, lower anxiety and a lower number of neurotic signs, but the cross-sectional design of the study made direct causal interpretations difficult.

Electroencephalograms (EEGs) were recorded from the Finnish workers who showed behavioral symptoms (96/98 of the workers) in the study by Seppalainen and Harkonen (1976). Abnormal EEGs were found in 23/96 (24%) of the workers, which was significantly higher than expected for the general population (10%). A dose-response relationship was indicated because subjects with abnormal EEGs had higher mandelic acid concentrations than subjects with normal EEGs; analyzing the data another way, workers with high styrene exposure had a significantly greater number of abnormal EEGs, whereas workers with lower exposure had a normal proportion of abnormal EEGs. Further analysis of these data (Harkonen et al., 1978) indicated that a marked change in the proportion of normal and abnormal EEGs occurred when the mandelic acid concentration was ≥700 mg/ℓ (estimated 8-hour TWA of 31 ppm). The proportion of abnormal EEGs reached a plateau at 800 mg/ℓ.

In case series studies, Klimkova-Deutschova (1962) and Roth and Klimkova-Deutschova (1963) also reported abnormal EEGs in 72% and 64%, respectively, of styrene-exposed workers. Their study populations were comprised of individuals who had reported to a clinic with subjective symptoms; no control group was used for comparison. Rosen et al. (1978) conducted EEG analyses of three exposed groups (exposure <5, 47 or 125 ppm TWA) and a control. No differences were observed in the alpha activity; the frequencies of increased fast activity and diffuse slow activity were somewhat different between groups, but no statistical analyses were performed to compare these frequencies. The exposed subjects were divided into two groups (with and without signs of polyneuropathy). An increased amount of fast activity was observed in 9/10 subjects in the polyneuropathy group compared with 1/22 in the other group.

Nerve conduction velocity studies have been performed by several investigators. Seppalainen and Harkonen (1976) found no significant differences for any of their nerve conduction measurements between a subgroup of 40 of the 98 Finnish laminating workers who had the most subjective complaints and an age-standardized control group.

Rosen et al. (1978) also found no differences in motor conduction velocity or distal motor latency between a control group and three styrene-exposed groups (exposures <5, 47 or 125 ppm TWA). However, signs of sensory neuropathy were indicated by significant alterations in the mean duration and amplitude of sensory action potentials in several of the exposed groups. The lengthened duration and reduced amplitude observed did not appear to be dose-related. The sample sizes, however (particularly of the control group), were small. A subgroup of 10 subjects with possible sensory neuropathy was compared with the remaining exposed workers (n=23). The group of 10 workers differed significantly ($p<0.05$) in all sensory neurography parameters tested (conduction velocity, amplitude and duration) as well as two motor neurography parameters (conduction velocity of ulnar nerve and distal latency). These 10 subjects had significantly higher styrene exposure and age than the rest of the group. An age correction did not affect the results.

Lilis et al. (1978) examined a subgroup (n=412) of the 488 workers surveyed by Lorimer et al. (1976, 1978) (discussed in the Subjective Effects Section). Workers with diabetes, histories of back injuries, or significant alcohol intake were eliminated from the study group. Prenarcotic symptoms were more prevalent in more highly exposed workers. Radial and peroneal nerve conduction velocities were less than the reference value in 19 and 16%, respectively, of the tested (80 and 73, respectively) workers. Peroneal nerve conduction velocity appeared to decrease as years of styrene exposure increased. No symptoms, disability or incapacitating peripheral neuropathy was observed.

Hepatic Effects. Serum levels of enzymes commonly used as indicators of liver dysfunction have been measured in workers occupationally exposed to styrene. The results are summarized in Table VI-2.

Chmielewski and Hac (1976) found no significant differences in SGOT and SGPT between short and long exposure groups; the levels were reportedly normal. Elevated SGPT was reported in 6/98 workers but this incidence was not compared with controls (Harkonen, 1977). Thiess and Friedheim (1978) found differences in the percentages of styrene workers (two groups) and controls (62 workers with similar working and living conditions) with abnormal SGOT, SGPT or GGT (percentage with abnormal GGT was higher in styrene group), but none of the differences were significant. Lorimer et al. (1976, 1978) also concluded that the incidence of abnormal SGPT, SGOT and GGT in styrene workers was not significantly different from a comparison group of 993 males aged 40-44 years. However, a high exposure subgroup did have a significantly higher prevalence of abnormal GGT than a low exposure subgroup (Lorimer et al., 1976, 1978). Axelson and Gustavson (1978) compared mean levels of SGOT and SGPT and found significantly higher levels in 35 styrene-exposed workers than in controls (12 male manufacturing workers).

Hotz et al. (1980) found that the activities of OCT, SGPT and GGT (but not SGOT) were weakly but significantly correlated with degree of exposure (see Table VI-2 for exposure schedule) measured as urinary concentration of mandelic and phenylglyoxylic acid (r=0.42, p=0.002 for OCT; r=0.40, p=0.003 for SGPT; r=30, p=0.02 for GGT). Degree of exposure had a greater effect on enzyme activities after a correction for alcohol consumption. Exposure duration was not correlated with enzyme activity, which is consistent with

TABLE VI-2

Hepatic Effects of Occupational Styrene Exposure

Study Population	Exposure Duration	Exposure Level	Effects	Reference
101 Polish styrene workers	~1 year average	~2.6 x MAC[a] (23.5-70.0 ppm)	AP, SGOT, SGPT, LDH, bilirubin, cholinesterase, cadmium and thymol hepatic tests reportedly normal. No significant difference between short and long exposure group. Levels of serum alpha and beta lipoproteins and cholesterol were significantly [illegible].001) lower in the long-employment group.	Chmielewski and Hac, 1976
21 Polish styrene workers	~10 years average	~6 x MAC[a] (70.0 ppm)		
98 Finnish male laminating workers	0.5-14 years (median 5.1 years)	median mandelic acid 808 mg/ℓ (range 7-4715 mg/ℓ)	Elevated SGPT (>40 μ/ℓ) in 6/98 workers	Harkonen, 1977
84 German male styrene workers	1-36 years	generally <1 ppm, (excursions to 10 ppm noted)	Percentage of styrene workers and reference workers with abnormal SGOT (>17 μ/mℓ), SGPT (>23 μ/mℓ) and GGT (>28 μ/mℓ) was 6.0 and 11.5, 10.7 and 23.0, and 27.5 and 13.3, respectively. These differences were not significant. No trend to higher values with age or duration of exposure. No apparent effect on LDH, AP, total bilirubin, total proteins, thymol, urea or creatine.	Theiss and Friedheim, 1978
93 German male polystyrene production workers	1-38 years	generally <1 ppm, (excursions to 8 ppm and higher noted)	Percentage of polystyrene workers and 62 reference workers with abnormal SGOT (>17 μ/mℓ), SGPT (>23 μ/mℓ) and GGT (>28 μ/mℓ) was 9.7 and 11.5, 22.6 and 23.0, and 21.5 and 13.3, respectively. These differences were not significant. No trend to higher values with age or duration of exposure. No apparent effect on leucinamino-peptidase, LDH, AP, total bilirubin, total proteins, thymol, urea or creatine.	Theiss and Friedheim, 1978
471 U.S. male styrene/polystyrene production workers (median age 40's)	~40% >20 years ~64% >7 years	generally <20 ppm TWA	Overall prevalence of abnormal AP (≥41 units), SGPT (≥70 I.U.), SGOT (≥50 I.U.), GGT (≥45 I.U.) and bilirubin (≥1.0 mg %) was in the range of 3-6% and not significantly different from a historical comparison group. High exposure (5-20 ppm) subgroup had a significantly ($p<0.02$) greater prevalence of abnormal GGT than a low exposure (<1 ppm) subgroup even when high alcohol consumers were excluded (7.2 vs. 1.7%).	Lorimer et al., 1976, 1978
35 Swedish laminating workers	NR	generally <100 ppm TWA	Mean levels of SGOT and SGPT were significantly increased when compared with 12 reference industrial workers ($p=0.001$ and $p=0.056$, respectively). No difference in mean levels of AP.	Axelson and Gustavson, 1978
57 Swiss polyester workers	~44% ≥5 years	<100 ppm TWA (estimated from metabolites)	Increased OCT, GGT and SGPT activity was correlated with degree but not duration of styrene exposure.	Hotz et al., 1980

[a] The mean urinary excretion of amygdalic acid in the short and long employment groups was 286.8 and 504 mg/ℓ, respectively (Chmielewski and Renke, 1976)

NR = Not reported

the finding of Thiess and Friedheim (1978). The mean enzyme activities were not markedly elevated in comparison with reference values, but the distribution of activities was shifted towards higher values in a subgroup (n=18) with 50-100 ppm estimated styrene exposures compared with a subgroup (n=30) with estimated exposure <50 ppm (Hotz et al., 1980).

Renal Effects. A series of reports summarized the kidney function of workers occupationally exposed to alkyl benzenes (Askergren et al., 1981a,b,c; Askergren, 1981). The exposed groups consisted of 101-134 men who had been exposed to styrene (plastic boat manufacturing), toluene (photogravure printing), and to xylene and toluene (paint manufacturing). Approximately one-third of the men were in the styrene-exposed group; 33-52 men were exposed to styrene in the two studies reporting numbers in each group (Askergren et al., 1981a,c). Employment duration was not reported. Unexposed control groups consisted of 39-48 male manual workers with mean age ~48 years (range 20-64) from the same geographical area. The controls were not characterized further. Detailed exposure data were not provided. One study reported that styrene levels ranged from 4.7-23.5 ppm, with some workers exposed to levels ≤217 ppm, but some workers were protected by gas masks or air-supplied respirators (Askergren et al., 1981a).

Askergren et al. (1981a) found that the mean urinary concentration of albumin and the number of subjects who excreted measurable amounts of albumin were significantly ($p<0.05$) higher in the styrene-exposed group (52 males, mean age 37±11 years) than in the controls (48 males, mean age 48±14 years). The urinary concentration of β-2-microglobulin was not significantly different between the two groups, which suggested to the authors that the renal damage was glomerular (increased permeability) rather than tubular.

Urinary excretion of erythrocytes and leukocytes was not reported specifically for styrene workers (Askergren, 1981). The group of 101 alkyl benzene-exposed workers (mean age 43±12 years) excreted significantly ($p<0.02$) more erythrocytes and leukocytes than the controls (mean age 48±13 years). The author reported that the differences between the styrene workers and the other organic solvent workers were not significant.

Renal concentrating ability was assessed by determination of urine osmolarity after 11 or 14 hours of fluid deprivation (Askergren et al., 1981b). Data were not reported specifically for the styrene group. No significant difference was found in the renal concentrating ability of the organic solvent workers (118 men, mean age 46±8 years) compared with 48 controls (mean age 48±14 years). This finding also indicates that the solvent-exposed workers did not have tubular damage (consistent with the results of normal B-2-microglobulin excretion) (Askergren et al., 1981a).

No significant difference in glomerular filtration rate (measured by plasma clearance of a single bolus injection of ^{51}Cr-EDTA) was observed between 33 styrene workers (mean age 34±8 years) and 48 male controls (mean age 48±14 years) (Askergren et al., 1981c).

Cytologic examination of the urine from 35 styrene workers revealed no atypical uroepithelial cells (Papanicolaou class 1) (Harkonen, 1977). The subjects were a subgroup of workers who had experienced abnormal psychological and EEG examinations from the group of 98 Finnish workers described in the Subjective Effects Section.

Hematologic Effects. Table VI-3 summarizes the design and results of the available studies assessing the hematologic effects of styrene on humans. A significantly lower number of platelets was found in 21 styrene workers (occupation not given) exposed to 6 times the maximal allowable concentration (MAC) for an average of 10 years compared with 101 styrene workers exposed to 2-6 times the MAC for only ~1 year average (Chmielewski and Renke, 1976). No significant effects were noted on erythrocyte or leukocyte counts or hemoglobin levels. A number of hematology parameters were significantly different in the long-employment workers compared with unexposed persons (see Table VI-3). Lorimer et al. (1976) reported incidences of abnormally low platelet counts, white blood cell counts and hemoglobin of 7.4, 2.7 and 13.6%, respectively, in a group of 486 styrene/polystyrene production workers exposed to generally 20 ppm TWA. These effects were not related to degree of exposure (4 ppm or 5-20 ppm) or employment duration. Relative lymphocytosis was diagnosed in 88/342 (26%) of these workers; the incidence increased with duration of exposure in the high exposure subgroup (5-20 ppm), but decreased with exposure duration in the low exposure group (4 ppm) and was not related to exposure intensity (Lorimer et al., 1978). The incidences of abnormal values for hemoglobin concentration, erythrocyte, leukocyte and platelet counts were determined for groups of 93 polystyrene and 84 styrene production workers and compared with a reference group. Styrene workers were exposed for 1-36 years to styrene vapor concentrations generally <1 ppm, but occasionally rising to near 10 ppm. The incidences of abnormal hematological parameters in these workers were similar to the reference group (Theiss and Friedheim, 1978).

TABLE VI-3

Hematologic Effects of Occupational Styrene Exposure

Study Population	Exposure Duration	Exposure Level	Effects	Reference
101 Polish styrene workers	~1 year average	~2.6 x MAC* (23.5-70.0 ppm)	Normal blood cell count and erythrocyte sedimentation in both groups, but a significant ($p<0.05$) reduction in platelet count was found in the long-employment group. Comparison of 21 long-employment workers and 20 normal persons showed significantly increased coagulation time (Lee White's method), reduced prothrombin ratio, reduced number of platelets, increased adhesion of platelets and decreased euglobulin fibrinolysis time; bleeding time (Dukes' method), level of fibrinogen, and aggregation of platelets did not significantly differ.	Chmielewski and Renke, 1976
21 Polish styrene workers	~10 years average	~6 x MAC* (70.0 ppm)		
486 U.S. male styrene/polystyrene production workers	~40% >20 years ~60% >7 years	generally <20 ppm TWA	Abnormal hemoglobin (<14.0 g%), white blood count (<4800) and platelet count (<18,000) in 13.6, 2.7 and 7.4% of the workers, respectively. Effect not related to high (5-20 ppm) or low (<1 ppm) exposure or duration of employment. Incidence of relative lymphocytosis (≥45%) was 47/164 (29%) in low exposure group and 41/178 (23%) in high group; overall incidence was 88/342 (26%). Lymphocytosis decreased with time in low group and increased in high group.	Lorimer et al., 1976, 1978
84 German male styrene production workers	1-36 years	generally <1 ppm (excursions to 10 ppm noted)	Percentage of styrene workers and 62 reference workers with abnormal hemoglobin (<13.6 g%), erythrocytes ($<4.5 \times 10^6$) and leukocytes (>9000) was 2.4 and 6.9%, 0 and 23.9% ($p<0.05$) and 17.9 and 33.5%, respectively. Thrombocyte count reportedly normal.	Theiss and Friedheim, 1978
93 German male polystyrene production workers	1-38 years	generally <1 ppm (excursions to 8 ppm and higher noted)	Percentage of polystyrene workers and 62 reference workers with abnormal hemoglobin, erythrocytes and leukocytes (criteria above) was 4.3 and 6.9%, 11.8 and 23.9% ($p<0.05$), 34.4 and 33.5%, respectively. Thrombocyte count reportedly normal.	Theiss and Friedheim, 1978
154 U.S. styrene-butadiene rubber manufacturing workers	NR	generally <1 ppm TWA except in the Tank Farm Dept. (mean ~14 ppm, 0.51-65.16 ppm). Concurrent exposure to butadiene, benzene and toluene	Styrene exposure had a very low negative correlation ($r = -0.178$) with erythrocyte count and a very low positive correlation ($r = 0.163$) with basophil counts. The small number of Tank Farm workers ($n=8$) precluded reliable statistical analysis, but comparison with all other workers showed slightly lower RBC, Hb, hematocrit, platelets and neutrophils, and slightly higher MCV, total WBC, band neutrophils, lymphocytes, eosinophils, basophils and monocytes. No difference in mean corpuscular Hb or reticulocytes.	Checkoway and Williams, 1982

*The mean urinary excretion of amygdalic acid in the groups with short and long employment was 286.8 and 504 mg/ℓ, respectively.

NR = Not reported

In the study by Chmielewski and Renke (1976) the styrene workers with reduced platelet counts (described above) also showed increased coagulation time and platelet adhesivity and reduced prothrombin ratio and fibrinolysis time when compared with an unexposed reference group. These effects have not been examined in other studies.

A cross-sectional survey was conducted on production workers at a U.S. styrene butadiene rubber manufacturing plant (Checkoway and Williams, 1982). Workers were exposed to styrene, butadiene, benzene and toluene at levels that varied in different departments. Eight-hour TWA exposures were <1 ppm in most areas, but styrene and butadiene exposures were much higher (13.67 and 20.03 ppm, respectively) in an area designated as the Tank Farm. The correlations (not adjusted for age or medical status) of styrene exposures with hematology parameters were significantly different from zero for two parameters. The correlation between red blood cell count and styrene exposure was very low and negative ($r = -0.178$, $p<0.05$) while a very low positive correlation ($n = 0.163$, $p<0.05$) was found between styrene exposure and basophil count. Differences in a number of parameters were noted between the Tank Farm and all other workers (see Table VI-3), but no statistical comparisons were made because of the small number of Tank Farm workers ($n=3$). The investigators concluded there was no striking evidence of abnormal hematology among the workforce tested.

Respiratory Effects. Lorimer et al. (1976, 1978) found significant differences between the high (5-20 ppm) and low (4 ppm) exposure groups with respect to acute lower respiratory symptoms in the health survey of 488 styrene production-manufacturing workers described in the Epidemiological

Studies Section. Indices of obstructive, restrictive and small airway dysfunction were also investigated, but the lack of comparison with an unexposed control group and concomitant exposure to butadiene and benzene make interpretation of these findings difficult. Nonsmoking workers with large airway obstruction (forced expiratory volume at 1 second [(FEV_1)/forced vital capacity (FVC) $<75\%$] had a significantly ($p<0.05$) higher prevalence of detectable urinary mandelic acid (12/29) than nonsmokers without obstruction (13/62) (Lorimer et al., 1978). Standard posterior-anterior chest X-rays of the workers (those without prior exposure to asbestos or coal dust) reportedly exhibited no noteworthy changes.

In a subgroup of 43/98 male laminating workers (median age 31 years, median exposures duration 6 years, median urinary mandelic acid concentration 472 mg/ℓ) no signs of pneumoconiosis were observed in chest X-rays and the results of lung function tests (FEV_1, FVC) were within normal limits (Harkonen, 1977). Chmielewski and Renke (1975) reported that 4/21 styrene-exposed workers (mean exposure duration 10 years, exposure level not well defined) had obstructive lung changes as measured by FEV_1/FVC. Gotell et al. (1972) found no change in vital capacity or FEV_1 before or after a workday in 17 fiberglass/polyester plastic workers (median age 28 years, exposure duration ≤ 12 years).

Jedrychowski (1982) compared 454 male employees from a styrene-methyl methacrylate plant with 683 reference males from a carbon derivative plant (with exposures to phenol, methanol and carbon monoxide). The mean concentration of styrene was 0.62 ppm (range 0.01-7.5 ppm) and the mean concentration of methyl methacrylate was 2.7 ppm (range 0.05-93.4 ppm). The mean age, mean employment durations and percentage of smokers were 36 years, 7 years and 68% in the styrene group and 41 years, 12 years and 60% in the reference group. The prevalence of chronic bronchitis and asthmatic symptoms were similar between the exposed and control group when total numbers, smokers or nonsmokers were compared. The frequency of lung obstruction (FEV_1 observed/FEV_1 expected <80%) was significantly ($p<0.05$) higher among exposed workers (45.5%) than the reference group (18.0%). This result cannot necessarily be attributed to styrene effects only. Additional analyses indicated that the effects on the development of lung obstruction of styrene/methyl methacrylate exposure and smoking were independent.

Reproductive Effects. The frequency of spontaneous abortions among Finnish chemical workers was analyzed by Hemminki et al. (1980a). Information on spontaneous abortions (15,482 cases), induced abortions (71,235 cases) and births (193,897 cases) for 1973-1976 was obtained from the Hospital Discharge Registry of the Finnish National Board of Health and linked by social security number to the membership registry of the Finnish Union of Chemical Workers (~900 female members). About 85% of the total number of spontaneous abortions in Finland were reportedly listed in the registry. The rate of spontaneous abortion was defined as the number of spontaneous abortions x 100/number of pregnancies and the ratio of spontaneous abortion was defined as the number of spontaneous abortions x 100/ number of births.

The rates of spontaneous abortions were 8.54 and 15.0% among the female union members (52 spontaneous abortions) and a subgroup in the styrene industry (6 spontaneous abortions), respectively (Hemminki et al., 1980a).

These rates were significantly higher ($p<0.01$) than the rate among all Finnish women (5.52%; 15,482 spontaneous abortions). The ratios of spontaneous abortion that, in this study, were defined differently than the rates, were 15.57 and 31.59% in the female union workers and female styrene industry workers, respectively, which were significantly higher ($p<0.001$) than the ratio among all Finnish women (7.98%).

Harkonen and Holmberg (1982) obtained the obstetric histories of 67 female lamination workers (mean age 30± years, range 19-40 years) exposed to styrene and 67 age-matched female textile and food consumption production workers of similar social class (mean age 31±5 years) with no obvious chemical exposures. The median duration of exposure was 4.5±2.6 years (range 0.5-10 years); exposure levels were not measured but the authors noted that the median TWA concentration of styrene in lamination work was 66 ppm in a previous study of the Finish polyester plastics industry. There were no significant differences in the number of women with pregnancies or spontaneous abortions between the exposed and reference group either before or during the period of styrene exposures. The number of births was significantly ($p<0.01$) lower among the exposed group during the styrene exposure period, but this difference could be explained by a higher number of induced abortions.

The information on the work histories of 43 Finnish mothers of children born with CNS defects from June 1, 1976 to March 1, 1977 were obtained through personal interviews (Holmberg, 1977). Two of the case mothers had been employed in the reinforced plastics industry with regular exposure during pregnancy to styrene, polyester resin, organic peroxides and acetone.

Exposures were verified by visits to the industries concerned; exposure levels were not quantified. The two defects were anencephaly and congenital hydrocephaly. The author estimated that the number of births expected in the female reinforced plastic industry workers of ages 18-44 during the 9-month study period would be ~12, based on the Finnish national fertility rate for 1974. The rate of anencephaly and hydrocephaly was reported to be 0.2 and 0.3, respectively, per 1000 live births in Finland. Based on these estimates there appeared to be more than a 300-fold increased rate of these malformations in the reinforced plastic industry workers during the 9-month study period compared with the general population (2/12 vs. 0.5/1000). It should be noted, however, that the results are based on a very small sample size.

Lemasters et al. (1987) studied birth outcomes of 1535 women, which was 70.5% of a cohort of women employed at least 6 months in companies that manufacture reinforced plastics. Of these, 1050 worked in jobs with exposure to styrene and 485 served as unexposed controls. Each woman contributed only one birth so that the fiscal sample was 230 exposed and 816 unexposed births. Data were collected by interview using a standardized reproductive and work history questionnaire. Exposure was constructed based on industrial hygiene exposure information, processes and job titles (Lemasters et al., 1985a). Seventy-five women were exposed to mean levels of 30-82 ppm including an average of 5.6 months of gestational exposure. The range of mean exposure level in the group with lower exposure was 2-2.9 ppm for an average 5.9 months during gestation. Three exposure indices were used: categorized (none, <30 ppm, $\geq$30 ppm in any one month of pregnancy), cumulative and critical period versions of the cumulative.

Analysis of effects of exposure on length of gestation showed no statistically significant effect in either of the statistical methods used. Least squares multiple regression showed no significant effect for birth weights whether adjusted or not adjusted for length of gestation. A trend of decreasing average birth weights with exposure was observed but did not test statistically significant. Birth weights in the 50 offspring of these highly exposed women was 3% lower than the weight for unexposed births. The analysis controlled for known covariates of low birth weight such as maternal age, cigarettes per day and alcohol consumption. These results, therefore, are suggestive of an effect. Also, potential biases listed that could result in underestimation of effect were that there were only 50 pregnancies in the most highly exposed jobs and many women were unexposed during the time when the fetus begins significant weight gain. Lemasters et al. (1985b) had evaluated menstrual disorders in 174 eligible exposed women in this cohort and 449 unexposed controls. The questionnaire was administered by telephone using trained interviewers. Menstrual dysfunction was defined as dysmenorrhea, intermenstrual bleedings secondary amenorrhea, menstrual blood clots and hypermenorrhea. Logistic regression analysis identified age, marital status, parity and presence of disease as risk factors but no effect on menstrual function was found for employment status or exposure.

Carcinogenicity. Retrospective cohort mortality and case-control studies have been conducted on workers exposed to styrene in the styrene-polystyrene manufacturing industry and in the styrene-butadiene synthetic rubber industry to assess the carcinogenicity of styrene. There are inadequate data at present to indicate that styrene is a human carcinogen.

However, an elevated incidence of cancers of the hematopoietic and lymphatic tissue has been observed. The available studies are limited because of relatively small cohort sizes or multiple chemical exposures of the workers (including exposure to benzene, a known leukemogenic agent).

McMichael et al. (1976) conducted a retrospective cohort study of 6678 male workers in a tire manufacturing plant. The mortality rates from 1964-1973 were determined for workers exposed between 1940 and 1960. The group of workers was subdivided using 16 job classifications; only 2-3% of the workers were employed for >2 years in the synthetic elastomer plant where styrene-butadiene rubber was made. These synthetic plant workers had risk ratios of 6.2 for lymphatic and hematopoietic cancer, 3.9 for lymphatic leukemia, and 2.2 for stomach cancer. Elevated risk ratios for these cancers were observed in several of the other job categories where there was no styrene exposure. In addition, Smith and Ellis (1977) noted that the relative risk estimate of 6.2 for lymphatic and hematopoietic cancer was based on only four deaths. In a follow-up unpublished case-control study cited by Meinhardt et al. (1978) the relative risk of lymphatic and hematopoietic cancer was reduced from 6.2 to 2.4, which was reportedly still a statistically significant increase.

The National Institute for Occupational Safety and Health (NIOSH, 1976) held a briefing on April 30, 1976 to review the hazards of styrene-butadiene production. Five cases of leukemia in a B.F. Goodrich styrene-butadiene rubber plant were described by Lemen and Young (1976). The leukemias were of diverse histologic types and there was no apparent association with a specific job classification. Three cases of leukemia in workers in a U.S. chemical synthetic rubber plant were also described (Lemen and Young, 1976).

Block (1976) described a study of six chemical plants in the Kentucky area including one synthetic rubber plant. Of the 72 death certificates obtained for workers employed between 1950 and 1975, leukemia was indicated as the cause of death in two cases and Hodgkin's disease was the cause in another two cases. An additional leukemia death was reported for a worker in 1976 (after the study cutoff date). There were three deaths from leukemia reported in the other five chemical plants with no styrene or butadiene exposure. IARC (1979) reviewed these reports and concluded the data were inadequate to indicate an association between an increased risk of leukemia and styrene or butadiene exposure; however, further study was justified.

NIOSH initiated a retrospective cohort mortality study of two styrene-butadiene rubber plants (designated A and B) to provide more substantial health hazard data (Meinhardt et al., 1978). This study is the most extensive epidemiological investigation of styrene to date. Meinhardt et al. (1982) reported the final results of this study. Records for Plant A covering the period 1943-1976 indicated 1662 white males were employed for ≥ 6 months (mean employment duration = 9.48 years), while 1094 white males were employed in Plant B during the period 1950-1976 (mean employment duration = 10.8 years). In Plant A the mean concentration of styrene, butadiene and benzene (the known leukemogenic agent) were 0.94, 1.24 and 0.1 ppm, respectively. In Plant B mean styrene and butadiene concentration were 1.99 and 13.5 ppm, respectively. These exposure levels may not reflect past exposures because of changes in the production process since the 1940s and 1950s. The expected number of cause-specific deaths were determined from the age, race, sex, calendar time of death and cause-specific mortality rates of the U.S. population. The standardized mortality ratio (SMR) was calculated from the observed and expected deaths. In plant A there were nine cases of neoplasms of the lymphatic and hematopoietic tissue (out of 252 total deaths) that resulted in an SMR of 155, which is not a statistically significant increase. The reevaluation of the data using a subgroup of white males (n=600, 201 had died) employed between 1943 and 1945 was prior to the discontinuation of the batch method of manufacturing. The SMRs for this subgroup were 212 for overall lymphatic and hematopoietic malignancies (p<0.1) and 278 for leukemia and aleukemia (p<0.1). In Plant B, 2/80 deaths were caused by neoplasms of the lymphatic and hematopoietic tissues, which yielded an SMR of 78. The healthy worker effect was used to explain the SMRs <100 that were observed for many of the causes of death. The authors noted that because of the modest sample size and the relatively high mortality rate for leukemia in the general population (8.8/100,000) an expected occurrence would have to be ~4 times larger than the observed occurrence to be a statistically significantly difference. In addition, the analyses were performed using the consecutive two-sided test (based on the Poisson distribution) customarily used by NIOSH. Using the one-sided test (which the authors feel is more appropriate), the mortality from leukemia and aleukemia for the Plant A subgroup was significant increased at the p=0.05 level. The authors (Meinhardt et al., 1978) concluded that the findings were suggestive of a possible association between excess mortality from lymphatic and hematopoietic cancers and exposure in the styrene-butadiene industry.

Ott et al. (1980) reported the results of a Dow Chemical retrospective cohort mortality study of employees involved in the development and manufacture of styrene products. The cohort consisted of 2904 persons who had been employed for ≥1 year during the period from the initiation of operation (1937-1960, depending on the plant) until 1975. Exposure to multiple agents often occurred. Based on mortality data for white males in the general U.S. population, the SMR for lymphatic and hematopoietic cancers (excluding leukemia) was 132, and the SMR for leukemia was 176. When a comparison cohort was used (all the workers, including the study cohort, employed at Dow Chemical's Midland Michigan plant in 1954), there was a significant ($p<0.05$) difference between the observed deaths and the expected deaths based on the comparison cohort for leukemia (6 observed compared with 1.6 expected). When observed versus expected deaths were compared in specific work environment categories, there was a significant increase in lymphatic leukemia (4 observed deaths vs. 0.5 expected) in one category where workers were exposed to styrene, polystyrene dust, ethylbenzene, oligomers of styrene, inorganic colorants and various solvents.

Nicholson et al. (1978) studied a cohort of 560 male employees from a styrene-polystyrene manufacturing plant who had at least 5 years seniority in 1960. Exposure to styrene was low (<1 ppm) for 116 workers; exposure was generally 5-20 ppm with some wide excursions for the remaining employees. Benzene and ethylbenzene exposure also occurred at unspecified levels. As of 1975, 83/560 workers had died. Fewer deaths were observed than expected (based on the general population) in all categories (including cancer).

These findings were attributed to the healthy worker effect. No difference in observed versus expected deaths was seen when the cohort was divided into high and low exposure groups. Of the 83 deaths, there were 2 leukemias and 1 lymphoma; an additional leukemia and lymphoma were reported among 21 deaths from workers who did not meet the criteria for inclusion in the cohort. The authors concluded that although there were no significant increases in cause-specific mortality, the incidence of leukemia (5/104 deaths) indicated a need for further study.

No increase in cancer deaths was found in styrene/polystyrene workers in the BASF Ludwigshafer manufacturing plant (Frentzel-Beyme et al., 1978). The cohort of 1960 workers was divided into workers employed before 1960 and workers employed after 1960 when the plant was modernized, resulting in lower exposures. The cohort was subdivided further into employees with <5, 5-10, 10-15 and >15 years of employment. The styrene concentration in 1975 was generally <1 ppm, with some samples as high as 8 ppm (Thiess and Friedheim, 1978). Fewer total deaths occurred in the exposure group than expected based on a comparison with 1970-1975 statistics from the local population (Frentzel-Beyme et al., 1978). There were also fewer deaths from malignancies than expected. There were increases in rare tumors when the cohort was subdivided into 5-year age classes, but these increases were not considered related to styrene exposure because they occurred sporadically in single age groups. The only statistically significant elevation in observed versus expected deaths in the subgroups based on length of exposure before and subsequent to 1960 was in the group exposed for 5-9 years after 1960. Since this elevation was only seen in one group and was not related to extent or duration of exposure, this result was considered an artifact.

Hardell et al. (1981) conducted a matched case-control study of all males ages 25-85 admitted to the Department of Oncology in Unea between 1974 and 1978 with malignant lymphomas. The control group consisted of 8 sex-, age- and residence-matched individuals for each living patient and 10 similar controls for each deceased case. Exposure histories were determined by questionnaires and a subcategory was provided for individuals exposed to styrene, benzene, trichloroethylene and perchloroethylene. One case and 5 controls were exposed to styrene, 7 cases and 3 controls were exposed to trichloroethylene, and 1 case each were exposed to perchloroethylene and benzene. The relative risk for developing malignant lymphomas was elevated (4.6; 95% confidence limits 1.9-11.4) in the subcategory that included all the above solvents. Analysis by individual solvent exposure was precluded by the small sample size.

An increase in proportional mortality ratios for digestive system cancers in styrene/polystyrene workers was reported by Liebling et al. (1982) in an abstract. No further information was available.

Hodgson and Jones (1985) conducted a mortality study of 622 men who worked for at least 1 year in the production, polymerization and processing of styrene at a chemical site in the United Kingdom during the period 1945-1974. This included 131 men who were potentially exposed to styrene, among other chemicals, in labortories and 491 individuals who would have had mixed chemical exposures but had specific potential exposure to styrene in the production of styrene monomer, the polymerization of styrene, or the manufacture of finished products. Mortality was surveyed until the end of 1978. The mortality of 3072 male manual workers who worked for at least 1 year at the same site during the same period but who were unexposed to styrene was also surveyed until the end of 1978. Two exposed persons and 181 unexposed persons could not be traced because of insufficient details in their personnel records. Four exposed persons and 181 unexposed persons were traced up until the time of their emigration. Causes of death were coded to the ICD revisions appropriate to the time of the study which included the fifth, sixth, seventh and eighth revisions. Incident cancer cases for the exposed population, but not the unexposed population, were obtained from the Regional Cancer Registry for the period January 1, 1962 to December 31, 1981. Expected mortality was calculated using the appropriate mortality rates for England and Wales. For the period 1967-1978, mortality rates for 10-year age groups were available for the northwest region of England where the styrene plant was located; these rates were also used to calculate expected deaths. Expected incident cases were calculated by applying age-specific registration rates to the person-years at risk of the exposed population. The rates were calculated by relating the Regional Cancer registrations to the population of the northwest hospital region.

The authors reported that there were 3 deaths from lymphoma in the exposed group where only 0.56 would have been expected based on mortality for England and Wales. This result is statistically significant ($p=0.02$). Deaths from causes other than lymphoma were not reported to be significantly in excess. Two of the lymphoma deaths occurred in the age group 15-44 (expected = 0.27, $p=0.032$), and one lymphoma death occurred in the 55- to 64-year-old age group (expected = 0.08, $p>0.05$). No deaths from leukemia were reported for the exposed group. The number of deaths from lymphoma was not significantly different from what would have been expected in the unexposed group; deaths from other causes were also not significantly in excess. There were no deaths from leukemia in the unexposed group.

Three cases of lymphoma and one case of leukemia were identified by the Cancer Registry for the exposed group. The three lymphoma cases were the same as the ones identified by the mortality study. In addition to the leukemia case, it should also be noted that one person in the exposed group who had died had leukemia listed as a secondary cause of death. The "standardized registration ratio" (SRR) for the three lymphoma cases was 375; the expected number of cases was 0.8 (p=0.047).

Independent pathologic evaluation by three pathologists of slides from the lymphoma cases identified all three as having non-Hodgkins lymphoma. Two of the cases were identified as centroblastic centrocytic follicular lymphoma. The third case was variably classified as a plasmablastic lymphoma, a B-cell lymphoma with plasmacytoid cells, and a follicular centroblastic centrocytic lymphoma.

A comparison by length of exposure between cases and referents (noncases with styrene exposure) whose age was within a year of the age of the case at the start of exposure did not demonstrate any association between duration of exposure and risk of being a case. The authors indicate that duration of exposure may not be a good index of exposure for the styrene workers since there may have been isolated excursions to higher levels associated with certain faults and some maintenance work.

Qualitatively the results of this study do provide some evidence for an association of styrene and the risk of lymphoma or leukemia. These results are consistent with the findings from other studies (McMichael et al., 1976, Ott et al., 1980). It also should be noted that at least two and possibly all three of the lymphoma cases found in this study were of the same histologic type. In addition, there was no excess cancer mortality among the group of workers at the plant not exposed to styrene.

Although the authors reported no association between duration of exposure and being a lymphoma case, they indicated that the variability of exposure intensity during a worker's employment history may make length of service a poor indicator of exposure. It also should be noted that the authors' comparison of exposure between cases and referents may have been biased since they did not match the cases and referents on calendar year of first exposure. Starting work in different calendar years could have had an effect on cumulative exposure.

The authors may also have biased the duration of exposure results by their matching criteria in another way. The authors' criteria for matching referents to cases was that "the referent's age at his start of service should be within ±1 year of the case's age at his start of service and that the referent should have survived alive and cancer-free (no registered cancer) for at least as long after start of service as the case". It is unclear from this definition whether the authors may have counted employment time for referents beyond the time period from first exposure to death from cancer (or cancer registration in the case of the leukemia victim) of the matched case. For example, case number 1 began exposure at 43.4 years, worked until about age 50, and died of lymphoma at age 62. Because the authors stated that the referents had to have survived at least as long after start of service as the case, it is possible that the authors may have continued to count employment for those referents to case number 1 who worked beyond 16.6 years (16.6 years is the time from beginning of exposure to the time of death for case number 1). If this were true, it would have biased the duration of employment toward the referents.

It is noteworthy that all three lymphoma cases and the one leukemia case were engaged in jobs involving styrene contact during the time period November 1960 to March 1961. The author mentions this fact because of some evidence linking social contact to leukemia risk. This fact is also interesting with regard to styrene exposure, however. Because employment duration was relatively short in all four (1.3-6.5 years) it is possible that an accident at the plant may have occurred during this time period resulting in elevated styene exposure and elevated cancer risk. The U.S. EPA's Carcinogen Assessment Group pursued this hypothesis with the authors and were told that nothing could be found in company records to suggest that a peak accidental exposure may have occurred during that time frame (November 1980 - March 1981). The authors did indicate, however, that relatively low level excursions above background could not be ruled out.

Finally, it should be noted that all of the cases were reported to have been exposed to chemicals other than styrene, some of which are known or suspected carcinogens. Also, the numbers on which the elevated risks are based are small. Thus, the study would be considered as providing some albiet inadequate evidence of a causal association between styrene exposure and an elevated risk of lymphoma.

In conclusion, several studies appear to show an association between styrene exposure with an elevated risk of leukemia and lymphoma in several epidemiologic studies. These results are confounded, however, by the exposures to multiple chemicals, some of which are known or suspected carcinogens, which took place in all of the studies. Following the Agency's Guidelines for Carcinogenicity, the epidemiologic evidence for a causal association would have to be considered inadequate.

Occupational Studies of Cytogenetic Changes

Chromosomal damage in peripheral lymphocytes has been studied in workers exposed to high levels of styrene in the production of polyester plastic products and to low levels of styrene in styrene and polystyrene manufacturing. The results of the studies, which are summarized in Table VI-4, indicate that an increase in chromosomal damage resulted from human exposure to high levels of styrene. It should be noted that workers in the industries listed in Table VI-4 are often exposed to chemicals other than styrene, including styrene oxide (very low levels), organic peroxides, solvents such as methylene chloride and acetone, and epoxide resins (Fleig and Thiess, 1978).

Lymphocytes isolated from 10 men employed from 0.5-8.5 years in the laminated plastic industry were examined for clastogenic effects and compared with those from 5 unexposed controls (Meretoja et al., 1977). The exposed workers showed an increase in interphase cells with micronuclei compared with controls (8.8% in exposed; 0.8% in controls) and an increase in chromosomal breaks (16.3% in exposed; 1.6% in controls). No statistical

TABLE VI-4

Cytogenetic Changes in Lymphocytes Isolated from Workers Occupationally Exposed to Styrene

Type of Industry	Number of Subjects		Exposure Levels[a]	Mean Number of Years Exposed	Results of Study			Reference
	Exposed	Control			Chromosomal Aberrations	SCEs	Micronuclei	
Reinforced plastic	10	5	1-300 ppm	3.2	+	ND	+	Meretoja et al., 1977
Reinforced plastic	16	6	1-300 ppm	6.3	+	-	ND	Meretoja et al., 1978b
Polyester resin plant	14	20	<50-300 ppm	7.9	+	ND	ND	Fleig and Thiess, 1978
Reinforced plastic	36	37	12-672 mg/m³ (2.8-154 ppm)	5	+	+[b]	ND	Andersson et al., 1980
Reinforced plastic	25	22	30 to >400 mg/m³ (7 to >92 ppm)	9.4	+	+	ND	Camurri et al., 1983
Reinforced plastic	6	6	60-310 mg/m³ (14-74 ppm)	4	+	ND	ND	Hogstedt et al., 1979
Reinforced plastic	16	13	33 to <70 ppm	ND	-	-	ND	Watanabe et al., 1981
Polystyrene pilot plant	24	24	6-58 ppm	14.4	-	ND	ND	Thiess et al., 1980
Styrene manufacture	5	20	<1 ppm[c]	21.6	-	ND	ND	Fleig and Thiess, 1978
Polystyrene manufacture	12	20	<1 ppm	20.3	-	ND	ND	Fleig and Thiess, 1978

[a] Exposure levels expressed as TWA when data were available. The ppm figures presented in parentheses were calculated using the formula: ppm = (mg/m³ x 24.45) ÷ 104.14.

[b] Only 21 exposed and 20 control subjects were used to determine SCEs.

[c] Estimated from a comparison of mandelic acid levels in this group and another group reported in the same paper for which exposure data were provided.

ND = No data

test results were reported. There was no marked association between urinary mandelic acid levels at the time of the analysis and number of aberrant cells.

In a subsequent report, Meretoja et al. (1978b) discussed chromosome aberrations in lymphocytes of 16 workers in the reinforced plastic industry and 6 unexposed controls. Five of the exposed workers and five of the controls were the same as in the previous study. Air samples and urinary mandelic acid measurements indicated that styrene exposure was as high as 300 ppm at times. There was a significant increase in chromosomal aberrations in exposed workers (mean 15.1±4.8%) compared with controls (2.0±1.3%). A similar incidence of chromosomal aberrations (mean 16.2%) was observed in a reexamination of 10 of the exposed workers a year later. The data were too limited to draw conclusions about the degree and duration of exposure in relation to the frequency of chromosomal aberrations. Cytogenetic damage as indicated by sister chromatid exchanges (SCE) was not significantly elevated.

The level of chromosomal aberrations in 6 male workers (ages 21-56) employed at a boat laminating plant for 0.5-10 years were compared with those of 6 age-matched, unexposed controls (Hogstedt et al., 1979). The TWA levels of styrene were determined to be 60, 310 and 115 mg/m³ (14, 74 and 27 ppm) in 1973, 1976 and 1977, respectively, with occasional peak levels up to 800 mg/m³ (188 ppm). The largest differences were in total aberrations (10.8% in exposed vs. 5.2% in controls) and in levels of chromosomal gaps and breaks (10.2% in exposed vs. 4.9% in controls). There was no marked relation between exposure duration and total aberrations.

Andersson et al. (1980) studied chromosomal aberrations in 36 exposed and 37 unexposed workers and SCE in 21 exposed and 20 unexposed workers. The workers were subdivided into high and low exposure groups based on cumulative styrene exposure determined by breathing zone measurements taken periodically from 1973-1978. There was a significant ($p<0.001$) increase in chromosomal aberrations in cultured lymphocytes from exposed workers (~8%) compared with controls (~3%). The incidences of chromosomal aberrations did not differ significantly between the high and low exposure groups. The frequency of SCE was increased ($p<0.05$) in the exposed group (mean = 8.4 SCE/cell) compared with the controls (mean = 7.5 SCE/cell).

Camurri et al. (1983) compared 25 workers from six different reinforced plastic industries with 22 controls matched on age, sex and smoking habits. A minimum and maximum level of styrene was determined in each plant twice a year. The exposure levels were 30-40, 70-100, 150-200, 200-250, 300-350 and >400 mg/m³ (7.0-9.4, 16.5-23.5, 35.2-47.0, 47.0-58.8, 70.4-82.3 and >94 ppm) for the six plants. Chromosomal aberrations were significantly ($p<0.005$) elevated in the workers at all plants compared with their controls. The average percentage of lymphocytes with aberrations were 30, 20, 32, 39, 37 and 44% in order from lowest to highest styrene exposure; the average percentages in the control groups ranged from 4.5-9%. There was a less marked elevation in SCE frequency with significant ($p<0.05$) increases observed in all groups except the lowest and fourth lowest exposure groups. The authors concluded that chromosomal aberrations were more sensitive indicators of styrene-induced chromosomal damage than SCE.

In contrast, Watanabe et al. (1981) detected no increases in chromosomal aberrations or SCE in 16 workers exposed to levels of styrene comparable with those in the above two studies. The average styrene levels were ~33 ppm in one plant and varied from 2-43 to 104-211 ppm with an estimated TWA exposure of <70 ppm in another plant. The only differences detected between the exposed workers and age and sex-matched controls were an inhibition of growth in the cultured lymphocytes and a slight decrease in the mitotic index.

Fleig and Thiess (1978) found a significant (p value not given) increase in aberrant lymphocytes in 14 workers at one plant with styrene levels reported by Thiess and Friedheim (1978) of <50-300 ppm when compared with unexposed workers at the same plant. No increase in aberrant cells was detected in workers from two industries with low levels of styrene exposure (Fleig and Thiess, 1978). In the polystyrene manufacturing plant Thiess and Friedheim (1978) reported styrene levels generally were <1 ppm, while low levels of exposure were indicated in the styrene manufacturing plant since only 2/5 workers studied had detectable levels of mandelic acid in their urine.

Thiess et al. (1980) examined lymphocytes from 24 workers in a styrene pilot plant. The employees were process workers exposed to an average of 58.1 ppm styrene and laboratory workers exposed to an average of 6 ppm styrene; numbers of workers in each group were not specified. The investigators reported that there was no significant increase in the frequency of aberrant cells either including or excluding gaps when exposed workers were compared with controls. However, Norppa et al. (1981) reanalyzed these data and found that the frequency of aberrant cells including gaps was significantly ($p<0.05$) or "almost" significantly higher in exposed workers compared with controls using several appropriate statistical tests (Chi-square test, Mann-Whitney U-test and the Fisher exact test).

High Risk Subpopulations

Persons with occupational exposure to styrene (e.g., in styrene-butadiene rubber plants, the reinforced plastic industry and styrene/polystyrene manufacturing) should be regarded as a special group at risk because they may be regularly exposed to elevated levels of this chemical.

Pregnant women may be a sensitive population. Fetotoxic effects have been indicated in laboratory animals (see Teratogenicity Section in Chapter V). There are equivocal results regarding increased spontaneous abortion rates in female styrene industry workers. Hemminki et al. (1980a) found a significant increase in the rate and ratio of spontaneous abortions in styrene industry workers compared with the general population, but there was no information on exposure levels or multiple chemical exposures. Exposure to a TWA concentration of 66 ppm styrene did not result in statistically significant increases in spontaneous abortion rates in 67 female lamination workers (Harkonen and Holmberg, 1982). Holmberg (1977) found that mothers of 2/12 infants born with CNS defects during a 9-month period experienced occupational exposure to styrene. The expected rate of CNS defects was 0.5/1000 births; based on the 9-month study period the authors estimated a rate of 2/12 births for styrene-exposed workers. These results suggest that the fetus may be more sensitive to styrene than are adults.

Sato et al. (1980, 1981) found ethanol both enhanced and inhibited styrene metabolism in rats depending on the dose level or the elapsed time since ethanol ingestion (see Synergism/Antagonism Section in Chapter VII). Coexposure to styrene and ethanol resulted in behavioral changes in rats suggesting a possible interaction between styrene and ethanol (Savolainin and Pfaffli, 1977). These results suggest that persons who consume alcohol regularly or excessively may be a sensitive population.

The effects of occupational exposure to styrene vapors have been the subject of a number of investigations for various endpoints including subjective, behavioral, neurophysiologic, hepatic, hematologic and reproductive.

Styrene exposure-related effects including prenarcotic symptoms, fatigue and mucous membrane irritation have been reported (Lorimer et al., 1976, 1978; Harkonen, 1977; Rosen et al., 1978). In comparison with controls, increased reaction times have been observed in workers exposed to styrene at concentrations $\geq$150 ppm for a few days to ~12 years (Gotell et al., 1972). This was not observed in workers exposed to lower styrene concentrations. Gamberale et al. (1975) noted increased reaction times at exposure levels of 13-101 ppm mean concentration and Kjellberg et al. (1979) observed altered reaction times at exposures below the Swedish TLV of 170 mg/m^3 at 11-60 mg/m^3 inspired air. Cherry et al. (1981) found a significant correlation between mandelic acid concentration and start-of-shift reaction time. Lindstrom et al. (1976) reported that altered psychological functions (particularly visuomotor accuracy) appeared to be related to styrene exposure. The threshold exposure for changes in visuomotor accuracy was estimated to be 800 mg/ℓ mean urinary mandelic acid concentration (~36 ppm

8-hour TWA) while a pronounced change in visuomotor and psychomotor performance occurred at >1200 mg/ℓ mandelic acid concentration (~55 ppm 8-hour TWA) (Harkonen et al., 1978). Lilis et al. (1978) observed prenarcotic symptoms and reduced nerve conduction velocity in workers exposed to styrene.

Summary

In controlled experiments with human volunteers, inhalation exposure to 376 ppm styrene for 1 hour resulted in altered neurophysiological function and eye and throat irritation (Stewart et al., 1968). Nasal irritation was observed after 20-minute exposures to 216 ppm, while no overt signs of toxicity were noted at exposure to 117 ppm for 2 hours (Stewart et al., 1968). Carpenter et al. (1944) observed behavioral effects and eye and throat irritation at a concentration of 800 ppm for 4 hours. Reaction time was significantly increased during the last of four 30-minute exposures to increasing concentrations of styrene (50, 150, 250 and 350 ppm) (Gamberale and Hultengren, 1974).

A significant increase in abnormal EEGs in styrene-exposed workers who show behavioral symptoms has been reported by Seppalainen and Harkonen (1976). A marked change in the proportion of abnormal and normal EEGs occurred when the mandelic acid concentration was >700 mg/ℓ (~31 ppm 8-hour TWA) (Harkonen et al., 1978). In a subgroup of this population, nerve conduction measurements were similar for exposed and control groups. Rosen et al. (1978) observed signs of sensory neuropathy (alterations in amplitude and duration of sensory action potentials) in three groups of workers exposed to <5, 47 or 125 ppm styrene. Lilis et al. (1978) found slowed radial and peroneal nerve conduction velocities in tested workers.

Results differ because exposure ranges, duration and sample size vary across experiments. The weight of evidence supports CNS effects in humans because the association increases with higher exposure and longer durations, and correlates with high mandelic acid concentrations in urine.

The activities of serum enzymes (SGOT, SGPT, GGT), which are indications of hepatotoxic effects, were reportedly normal in 101 styrene workers exposed to an average of 23-70 ppm (Chmielewski and Hac, 1976) and were not significantly different from controls in 84 styrene workers exposed to generally <1 ppm (Thiess and Friedheim, 1978). However, Axelson and Gustavson (1978) found significantly higher levels of SGOT and SGPT in workers with exposure to <100 ppm styrene than in controls. A group of workers exposed to 5-20 ppm styrene had a significantly higher prevalence of abnormal GGT than a group exposed to <1 ppm (Lorimer et al., 1976, 1978). Hotz et al. (1980) also noted that individuals exposed to 50-100 ppm tended to have higher serum enzyme activities than individuals with exposure to <50 ppm. The mean urinary concentration of albumin was significantly higher in the styrene-exposed workers than in controls, but indicators of renal tubular damage were negative (Askergren et al., 1981a,b) and the glomerular filteration rate was apparently not affected by styrene exposure (Askergren et al., 1981c).

Styrene appears to have minimal adverse hematologic effects. Chmielewski and Renke (1976) noted a significantly reduced platelet count in a group of styrene workers exposed to ~70 ppm for an average of 10 years compared with workers exposed for an average of 1 year. A number of other hematology parameters differed from those of a control group. Several other studies have reported results of hematology analyses in styrene workers

(Lorimer et al., 1976, 1978; Thiess and Friedheim, 1978; Checkoway and Williams, 1982) but low exposure levels and lack of comparison with controls makes interpretation of their results difficult.

There were significant differences between high (5-20 ppm) and low (<1 ppm) exposure groups with respect to acute lower respiratory symptoms (Lorimer et al., 1976, 1978). No marked differences in lung function test results were observed in styrene-exposed workers (Harkonen, 1977; Axelson and Gustavson, 1978). Chmielewski and Renke (1975) reported obstructive lung changes in 4/21 styrene-exposed workers but no comparison was made with controls.

Studies of possible reproductive effects of styrene are limited in number and design (small sample sizes; quality exposure data). Hemminki et al. (1980a) found the rate of spontaneous abortions in Finnish styrene industry workers was higher ($p<0.01$) than the rate among all Finnish women. The ratio of spontaneous abortions to number of births was significantly ($p<0.001$) higher in styrene workers. Spontaneous abortion rates did not differ between 67 controls and 67 lamination workers with styrene exposures estimated as 66 ppm (from previous study) (Harkonen and Holmberg, 1982). Mothers of 2/43 children born with CNS defects in Finland during a 9-month period were employed in the reinforced plastic industry with exposure to styrene (Holmberg, 1977). The authors estimated that 12 births would be expected among the styrene exposed workers, so a rate of 2/12 infants with CNS defects was high, compared with the expected rate of 0.5/1000.

Lemasters et al. (1985b) did not find statistically significant effects on menstrual function in 174 exposed workers compared with 449 controls. A dose-related trend to low birth weight in infants born to styrene workers did not reach statistical significance in a study of 1050 exposed workers from the same population (Lemasters et al., 1987).

There are inadequate data to indicate that styrene is a human carcinogen. Most of the available studies are limited because of multiple chemical exposures of the study populations or small cohort sizes. McMichael et al. (1976) found elevated risk ratios for lymphatic and hematopoietic cancer, lymphatic leukemia and stomach cancer in a styrene-butadiene rubber production plant, but these rates were based on a small number of deaths. Reports of cases of leukemia in styrene-butadiene rubber plants prompted NIOSH to initiate a retrospective cohort mortality study of workers in two styrene-butadiene plants. The results of this study indicate that the SMR for lymphatic and hematopoietic neoplasms was >100 for the group of workers from one of the plants (Meinhardt et al., 1982). A subgroup from this plant with higher styrene exposure (employed before manufacturing process changes) had SMRs of 212 for overall lymphatic and hematopoietic malignancies and 278 for leukemia and aleukemia. The elevations for leukemia and aleukemia were significant ($p<0.05$) using a one-sided test, but not using the two-sided test customarily employed by NIOSH (Meinhardt et al., 1978). Ott et al. (1980) calculated SMRs of 132 for lymphatic and hematopoietic cancers and 176 for leukemia in styrene-exposed workers. Leukemia was the cause of death for 5/104 deaths in styrene-polystyrene workers (Nicholson et al., 1978). Hodgson and Jones (1985) also found an association between styrene exposure and leukemia and/or lymphoma. Frentzel-Beyme et al. (1978) reported that the incidence of cancer deaths in styrene-polystyrene workers was not elevated. An elevated risk of digestive system cancers in styrene-polystyrene workers was reported in an abstract (Liebling et al., 1982).

An increase in chromosomal damage in peripheral lymphocytes has been observed in workers with occupational exposure to multiple chemicals including levels of styrene in the range of 1-300 ppm (Meretoja et al., 1977), <50-300 ppm (Fleig and Thiess, 1978), 2.8-154 ppm (Andersson et al., 1980), 7 to >92 ppm (Camurri et al., 1983) and 14-71 ppm (Hogstedt et al., 1979). Alterations in the frequency of chromosomal aberrations were not found in studies of workers exposed to 30 to <70 ppm (Watanabe et al., 1981), 6-58 ppm (Thiess et al., 1980) and <1 ppm (Fleig and Thiess, 1978).

Persons with occupational exposure to styrene may be a high risk subpopulation, but available data are not adequate to verify fetotoxic or teratogenic effects of styrene in humans.

VII. MECHANISMS OF TOXICITY

Metabolism

Styrene is oxidized to an intermediate metabolite, styrene oxide, by microsomal mixed-function oxidase. Styrene oxide is a reactive epoxide believed to mediate the observed toxic effect of styrene.

The pathways by which styrene oxide is detoxified have been studied by several investigators. Based on their findings, Watabe et al. (1978a) suggested that the relative ratios of cytochrome P-450 dependent monooxygenase (catalyzes conversion of styrene to styrene oxide) to epoxide hydratase (catalyzes conversion of styrene oxide to styrene glycol) will determine the availability of styrene oxide. The lung tissue in mice and rabbits has an unusually low epoxide hydrolase specific activity relative to monooxygenase and is particularly vulnerable to exposure by both styrene and the epoxide (Cantoni et al., 1978). Belvedere et al. (1977) suggested that epoxide hydratase is the rate-limiting enzyme in the metabolism of styrene since the hydratase had a higher affinity for styrene oxide than monooxygenase had for styrene. Detoxification of styrene oxide through binding with GSH to form glutathione conjugate has been demonstrated by reductions in hepatic GSH content in laboratory animals following administration of styrene oxide (Chakrabarti and Brodeur, 1981c; Vainio and Elovaara, 1979) or styrene (Parkki, 1978; Parkki et al., 1978; Vainio et al., 1979; Vainio and Makinen, 1977; Das et al., 1981a).

Covalent binding of styrene oxide to cellular macromolecules is a suggested mechanism of toxicity. Marniermi et al. (1977) detected covalent binding of styrene oxide to macromolecules in rat liver both _in vitro_ and _in vivo_. Intraperitoneal administration of 7-^{3}H-styrene oxide resulted in covalent binding of radiolabel to total liver homogenate and the microsomal fraction. One hour following treatment, 2-3% and 30% of the detected radioactivity from a 50 and 200 mg/kg dose, respectively, were covalently bound. Both crude protein and nucleic acid fractions contained bound styrene oxide. Styrene oxide has been examined for the ability to react chemically with DNA. Van Anda et al. (1979) detected covalent binding of radioactivity to DNA only at 500 μmol ^{14}C-styrene oxide/isolated perfused rat liver; binding was not detected at 100 μmol or less. In the study by Marniemi et al. (1977), ~12 and 5% of the nonextractable radioactivity was associated with nucleic acids following intraperitoneal administration of 7-^{3}H-styrene oxide at levels of 50 and 200 mg/kg, respectively. It was not determined whether binding was to RNA, DNA, or both. Covalent binding of ^{14}C-styrene and ^{3}H-styrene-7,8-oxide to DNA of various tissues has recently been demonstrated by Nordqvist et al. (1985) following i.p. injection in male NMRI mice.

Drinkwater et al. (1978) tested the ability of mutagenic compounds to react with supercoiled SV40 DNA, resulting in unwinding of the supercoils. No DNA unwinding was detected with styrene oxide concentrations up to the limits of solubility. Hemminki et al. (1980b) observed a very limited degree of reactivity when a high concentration (0.5 M) of styrene oxide was incubated with the four deoxyribonucleosides of DNA.

Styrene administration has been demonstrated to result in enzyme induction and inhibition in the liver, kidney, brain and lungs of laboratory animals. These effects are detailed in the General Toxicity Section and the Target Organ Toxicity Section of Chapter V. Enhanced activity of several mixed-function oxidase enzymes following styrene exposure were reported by

Vainio et al. (1979), Srivastava et al. (1982) and Das et al. (1981a, 1983). Styrene exposure resulted in enhanced epoxide hydratase activity (Parkki et al., 1976; Sandell et al., 1978a,b) and enhanced UDP-glucuronsyltransferase activity (Sandell et al., 1978a,b). Depressed glutathione-S-transferase activity has been observed after styrene exposure (Srivastava et al., 1982; Das et al., 1981a,b, 1983).

Ohtsuji and Ikeda (1971) found that pretreatment of rats with phenobarbital, a microsomal enzyme inducer, potentiated the formation of styrene oxide from styrene. Pretreatment with a microsomal enzyme inhibitor, SKF 525-A, inhibited styrene metabolism (Ohtsuji and Ikeda, 1971). Chakrabarti and Brodeur (1981a) reported that styrene alone significantly increased the levels of SGOT and SGPT in rats (indicators of hepatotoxicity), but these levels were not further enhanced by pretreatment with phenobarbital or 3-methylcholanthrene (microsomal enzyme inducers) or reduced by treatment with SKF 525-A. Parkki (1978) observed a reduction in the elevation in SGPT in hamsters following styrene administration with 3-methylcholanthrene pretreatment. Ryan et al. (1976) found that epoxide hydratase and glutathione-S-transferase activities towards styrene oxide were increased in the liver following phenobarbital pretreatment.

Treatment with methionine (a GSH precursor) resulted in a significantly lower level of SGPT in hamsters following a single intragastric dose of styrene, which suggested a protective effect of methionine (Parkki, 1978). Diethyl maleate (a GSH depletor) potentiated the hepatotoxic effect of styrene as indicated by SGPT activity in hamsters administered styrene (1 g/kg) for 5 days (Parkki, 1978).

The mechanisms for the CNS effects of styrene are not well characterized, but several investigations have noted biochemical changes in the brain following styrene exposure. Agrawal et al. (1982) reported a significant ($p<0.05$ or 0.01) increase in the binding of ^{3}H-spiroperidol to dopamine receptors in the corpus striatum of rats given single doses or multiple doses for 90 days of 200 or 400 mg/kg styrene. Behavioral changes could result from alterations in the dopaminergic system. Husain et al. (1980) found significantly ($p<0.001$) elevated levels of noradrenaline and serotonin, but dopamine levels were not altered in rats administered 910 mg/kg styrene for 15 days. Savolainen and Pfaffli (1977) noted a number of biochemical alterations in the brain after inhalation exposure of rats to 300 ppm styrene for 9-11 weeks. These changes included increased creatine kinase, acetyl cholinesterase and acid proteinase activity, increased RNA content and decreased protein content. The authors suggested that these changes may be early indications of neurotoxic action.

Synergism/Antagonism

Styrene metabolism and, therefore, toxicity may be affected by coexposure to other industrial chemicals, alcohol consumption or diet. Simultaneous administration of toluene (217 mg/kg) with styrene (223 mg/kg) to rats by intraperitoneal injection significantly ($p<0.05$) reduced the urinary concentration and delayed the excretion of the styrene metabolites, phenylglyoxylic and mandelic acid (Ikeda et al., 1972). The inhibitory effect of toluene on styrene metabolism was reduced with the coadministration of phenobarbital. These results suggested to the authors that coexposure to toluene and styrene may result in delayed styrene oxide detoxication because of competitive inhibition of metabolizing enzymes. Reduced urinary concentration of styrene metabolites was also observed with simultaneous inhalation exposure of rats to toluene (136-1333 ppm) and styrene (100-170 ppm) for 6 hours (Ikeda and Hirayama, 1978).

Ikeda and Hirayama (1978) also reported that coinjection of or coexposure by inhalation to trichloroethylene and styrene suppressed excretion of mandelic and phenylglyoxylic acid. Coadministration of other organic solvents (n-hexane, ethyl acetate, methylchloroform and acetone) did not affect the excretion of the urinary metabolites of styrene in a screening experiment (intraperitoneal doses of 2.2 mmol/kg of styrene and solvent) conducted by Ikeda and Hirayama (1978). Parkki et al. (1980) reported that cadmium binds noncompetitively to epoxide hydratase, thereby inhibiting the metabolism of styrene oxide.

Sato et al. (1980) noted that in vitro metabolism of styrene was increased ~3-fold when styrene was added to microsomes prepared from rats killed ~10 hours after termination of a 3-week exposure to ethanol (ingested in drinking water) compared with controls (no ethanol). Metabolism was not enhanced in microsomes from rats withdrawn from ethanol for 24 hours before sacrifice. Sato et al. (1981) subsequently studied the effect of ethanol on the metabolism of a number of hydrocarbons including styrene by administering ethanol by gavage in single doses of 0, 2, 3, 4 or 5 g/kg to rats that were sacrificed 18 hours later. The greatest ennancement of the in vitro metabolism of styrene by microsomes from these rats was observed at an ethanol dose of 4 g/kg; less enhancement occurred at 5 g/kg. At the time of sacrifice, relatively large amounts of ethanol remained in the liver and blood of rats dosed with 5 g/kg ethanol, while almost no ethanol was retained in rats given 4 g/kg. The metabolic rates of microsomal and ethanol-containing soluble fraction from 5 g/kg ethanol-treated rats and the microsomal fraction from 5 g/kg ethanol-treated rats mixed with the soluble fraction from control rats were compared. The metabolic activity was less in the presence of ethanol, indicating that ethanol inhibits enzyme activity. Metabolism of styrene was also inhibited when ethanol was added directly to the incubation mixture. Sato et al. (1981) suggested that ethanol both inhibited and enhanced drug metabolizing enzymes depending on the quantity of ethanol remaining in the body.

In humans exposed to 200 mg/m^3 (47 ppm) styrene for 6 hours, ethanol ingestion (0.45 g/kg) halfway through the inhalation exposure temporarily inhibited mandelic acid excretion (Wilson et al., 1983a). Phenylethane-1,2-diol, the direct metabolic precursor of mandelic acid, was identified in the blood after 4 hours of inhalation exposure to styrene, and its concentration increased 15-fold with ethanol administration (blood samples taken 1 hour after ethanol ingestion) (Wilson et al., 1983a).

The effects of protein, fat and carbohydrates on the metabolism of styrene were studied by varying their concentrations in test diets, preparing microsomal enzymes from the test rats and adding styrene as a substrate (Nakajima et al., 1982). Liver microsomal enzyme activities were increased by decreased food intake or decreased sucrose content. A carbohydrate-free diet (with high protein and fat) resulted in an increase in the rate of styrene metabolism comparable with 1 day of food deprivation. The authors concluded that a high dietary carbohydrate content resulted in a decreased rate of styrene metabolism.

Summary

Styrene is oxidized to styrene oxide, an intermediate metabolite that is highly reactive and is presumed to mediate the observed toxic effects of styrene. Styrene oxide is detoxified by conversion to styrene glycol (catalyzed by epoxide hydratase) or to glutathione conjugates (catalyzed by glutathione-S-transferase). The relative ratios of cytochrome p-450 dependent monooxygenase to epoxide hydrotase may determine the availability of styrene oxide. The mouse and rabbits lung tissue has been shown to have unusually low epoxide hydrolase activity relative to monooxygenase levels. Phenobarbital potentiates the formation of styrene oxide in rats (Ohtsuji and Ikeda, 1971) and also increases the activities of the enzymes that detoxify styrene oxide (Ryan et al., 1976). Hepatotoxicity is apparently not affected by two microsomal enzyme inducers (phenobarbital and 3-methylcholanthrene) or one inhibitor (SKF 525-A) in rats (Chakrabarti and Brodeur, 1981a). A GSH precursor (methionine) and a GSH depletor (diethyl maleate) inhibited and potentiated, respectively, the hepatotoxicity of styrene in hamsters (Parkki, 1978).

A suggested mechanism of toxicity is covalent binding of styrene oxide to cellular macromolecules, which has been observed by Marniemi et al. (1977) and Van Anda et al. (1979). Induction of mixed-function oxidases (Vainio et al., 1979; Srivastava et al., 1982; Das et al., 1981a, 1983), epoxide hydratase activity (Parkki et al., 1976; Sandell et al., 1978a,b) and inhibition of glutathione-S-transferase activity (Srivastava et al., 1982; Das et al., 1981a,b, 1983) have been observed following styrene exposure. Biochemical changes in the brain in response to styrene exposure have been noted by Agrawal et al. (1982), Husain et al. (1980) and Savolainen and Pfaffli (1977).

In vitro studies using cells in culture or isolated DNA have generally failed to demonstrate interactions of styrene oxide with DNA (Drinkwater et al., 1978). Van Anda et al. (1979) did detect covalent binding of styrene oxide to DNA in perfused rat liver and Hemminki et al. (1980b) demonstrated limited reactivity of styrene oxide with the deoxyribonucleosides of DNA. Covalent binding of ^{14}C-styrene and ^{3}H-styrene-7,8-oxide to DNA of various tissues has recently been demonstrated by Nordqvist et al. (1985) following i.p. injection in male NMRI mice.

Styrene metabolism was inhibited by coexposure to toluene (Ikeda et al., 1972; Ikeda and Hirayama, 1978) or trichloroethylene (Ikeda and Hirayama, 1978). Ethanol consumption appeared to both inhibit and enhance drug metabolizing enzymes in rats (Sato et al., 1980, 1981). Mandelic acid excretion was temporarily inhibited by ethanol ingestion in humans exposed to styrene vapors (Wilson et al., 1983a). Nakajima et al. (1982) found that styrene metabolism was inhibited by a high carbohydrate content in the diet.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Introduction

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \underline{\quad\quad} \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicological effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the U.S. EPA (1986a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{(\text{RfD}) \times (\text{Body weight in kg})}{\text{Drinking Water Volume in } \ell/\text{day}} = \underline{\qquad} \text{ mg}/\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{UF}) \times (\underline{\quad}\ \ell/\text{day})} = \underline{\qquad} \text{ mg}/\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

Styrene is not highly toxic following single inhalation or oral exposure. An LD_{50} of ~5000 mg/kg was reported for rats (Wolf et al., 1956), while LC_{50} values for rats and mice ranged between ~2400 and 4900 for 4- to 6-hour inhalation exposures (see Table V-1).

Human volunteers have been exposed to styrene vapors in controlled experiments. In an early study by Carpenter et al. (1944), a 4-hour exposure of two male subjects to 3407 mg/m³ (800 ppm) styrene resulted in drowsiness, listlessness, impaired balance, unsteadiness and eye and throat irritation. No other exposure levels were tested. Odkvist et al. (1982) studied the effects of styrene on the vestibulo-oculomotor function in 10 subjects exposed to styrene at concentrations between 87 and 139 ppm (370-591 mg/m³) for ~80 minutes. The rate of movement of the eyes between two alternating light sources (saccade) increased significantly ($p<0.05$)

after exposure, but rotatory and optokinetic nystagmus were not affected. Twelve subjects exposed to styrene concentrations of 213, 639, 1065 and 1491 mg/m^3 (50, 150, 250 and 350 ppm) during four consecutive 30-minute intervals exhibited a dose-related increase in simple reaction time (Gamberale and Hultengren, 1974). A significant increase in reaction time occurred during the final 30-minute exposure. Manual dexterity and perceptual speed were not significantly affected by styrene exposure. The altered reaction time observed in this study (Gamberale and Hultengren, 1974) occurred at a much lower exposure level than the signs of intoxication reported by Carpenter et al. (1944). Stewart et al. (1968) reported that volunteers exposed to mean levels of styrene of 51 ppm (217 mg/m^3) and 117 ppm (499 mg/m^3) for 1 and 2 hours, respectively, showed no signs of toxicity. Nasal irritation was reported in 1/3 subjects after 20 minutes of exposure to 216 ppm (921 mg/m^3). Exposure to a mean of 376 ppm (1602 mg/m^3) for 1 hour resulted in eye and nose irritation, strong odor and altered neurological function indicated by reduced performance in the modified Romberg test (1/5 subjects), the Crawford Manual Dexterity Collar and Pin Test (2/5 subjects) and the Flannagan Coordination Test (3/5 subjects). Six subjects were exposed to 99 ppm (422 mg/m^3) styrene vapor for two 3.5-hour periods separated by a 30-minute period without exposure. There were no objective signs of impaired balance or coordination during the 7 hours. The scores of the Flannagan Coordination tests and the Crawford Manual Dexterity test were unchanged from pre-exposure scores. Clinical laboratory studies following postexposure were normal and unchanged from the pre-exposure values.

Enzyme induction or inhibition has been reported in a number of short-term (7-day) oral studies using rats. Dixit et al. (1982) reported a significant ($p<0.001$) decrease in brain aryl hydrocarbon hydroxylase activity when styrene was administered for 7 days at a daily dose of 270 mg/kg. Changes in the activity of drug-metabolizing enzymes in the brain, kidney and liver have been reported following seven daily doses of ≥450 mg/kg styrene (Das et al., 1981a,b, 1983). Husain et al. (1980) observed a significant ($p<0.001$) increase in brain noradrenalin and serotonin, and a significant ($p<0.001$) decrease in brain monoamine oxidase activity in rats after 15 consecutive daily oral doses of 1 mℓ/kg (~900 mg/kg). No significant changes in body weight or brain weight were observed. In a 4-week oral study, Spencer et al. (1942) reported no apparent toxic effects or pathological lesions in rats dosed with 100 mg/kg/day for 5 days/week, reduced weight gain but no significant pathological or hematologic effects at a dose of 500 mg/kg/day and an unspecified number of deaths at 1000 mg/kg.

Effects of single exposures to styrene by inhalation include altered behavior (increased swimming time) in mice exposed to ≤851 ppm x 4 hours (De Ceaurriz et al., 1983) and weakness and unsteadiness in rats exposed to a high concentration (~1400 ppm) for ≤30 hours (Spencer et al., 1942). Altered activity of drug-metabolizing enzymes was observed in rats exposed to 450 ppm styrene, 8 hours/day for 7 days (Sandell et al., 1978a,b) and in rats exposed to 300 ppm, 6 hours/day, 5 days/week for 2 weeks; degenerative morphological alterations in hepatic parenchymal cells was also observed at the 300 ppm exposure (Vainio et al., 1979).

In a 90-day oral study with rats, daily doses of 200 or 400 mg/kg did not affect body weight or striatal weight (Agrawal et al., 1982). There was a dose-related increase in the specific binding of ^{3}H-spiroperidol to rat striatal membranes. There was a significant increase in binding following administration of styrene in a single oral dose of 200 mg/kg ($p<0.05$) or 400 mg/kg ($p<0.001$). No effect on body weight or striatal weight was seen at either dose. The authors (Agrawal et al., 1982) noted that this altered sensitivity of dopamine receptors has been observed with other neurotoxic chemicals. Oral administration of styrene to rats in a dose of 200 or 400 mg/kg/day, 6 days/week for 100 days resulted in dose-dependent changes in a number of drug-metabolizing enzymes (Srivastava et al., 1982). There were no significant differences in body weight gain or absolute or relative liver weights in treated and control animals, nor overt signs of toxicity in any of the styrene-exposed animals. At the higher dose (400 mg/kg/day), mild hepatotoxicity was indicated by significant ($p<0.02$) increases in SGOT and SGPT activities and by areas of focal necrosis. At 200 mg/kg, the levels of SGOT and SGPT were somewhat elevated over controls, but these increases were not significant and no liver necrosis was noted. A NOAEL of 200 mg/kg, 6 days/week (171 mg/kg/day, 7 days/week) is defined by this study.

Four inhalation studies have been conducted with rats exposed to 300 ppm styrene, 6 hours/day, 5 days/week for 4-13 weeks. Seppalainen (1978) reported a transient increase in the motor conduction velocity of the tail nerve of rats. Behavioral observations and biochemical analysis of cerebral glial cells revealed no significant effects in rats exposed for $\leq$13 weeks (Savolainen et al., 1980). In the rat brain, creatine kinase, acetylcholinesterase and acid proteinase activity and RNA content increased significantly after 9 or 11 weeks of exposure (but not <9 weeks), while protein content decreased significantly at week 9 (Savolainen and Pfaffli, 1977). Vainio et al. (1979) found minor histological alterations in rat liver parenchyma and induction of drug-metabolizing enzymes following exposures for ≤11 weeks.

In a chronic bioassay, Ponomarkov and Tomatis (1978) administered styrene by gavage to rats at a dose level of 500 mg/kg once a week for 120 weeks. Lesions of the kidney and forestomach, lung and kidney congestion and small necrotic foci in liver parenchyma were observed in some animals. Since the toxicokinetic data indicate that styrene is rapidly metabolized and eliminated from the body (see Chapter III), this dose schedule would presumably result in wide fluctuations in styrene concentrations in the body. Rats administered oral doses of styrene 5 days/week for 6 months exhibited depressed growth rates and increased liver and kidney weights at dose levels of 400 and 667 mg/kg/day (Wolf et al., 1956). No hematologic or histopathologic effects were observed. No body weight, organ weight or pathological effects were observed at doses of 133 or 66.7 mg/kg/day.

In a 2-year chronic toxicity study (Beliles et al., 1985) Sprague-Dawley rats were administered styrene in the drinking water at 0, 7.7 and 14 mg/kg/day for males and 0, 12 and 21 mg/kg/day for females. Except for statistically significant reduction in water consumption for styrene-treated rats, no treatment-related changes including mortality were observed.

In a subchronic study, beagle dogs administered styrene by gavage 7 days/week for 561 days showed no adverse effects at a dose of 200 mg/kg/day (body weight and organ weight measurements, urinalysis and hematological examinations were performed) (Quast et al., 1978, 1979). At 400 and 600 mg/kg, there were minimal histopathologic changes in the liver, increased numbers of Heinz bodies in red blood cells and a decrease in packed cell volume.

The effects of occupational exposure of humans to styrene have been studied extensively. Many of the occupational studies described in Chapter VI are limited by lack of precise exposure data, grouping of individuals with widely differing exposure durations, lack of controls, simultaneous exposure to multiple chemicals and cross-sectional designs. Equivocal results reported from investigations of hematological, hepatic and renal effects preclude the evaluation of health effects based on these studies. An increase in chromosomal damage in peripheral lymphocytes has been reported in workers with exposure to styrene in the range of 1-300, <50-300, 2.8-154, 7->92 and 14-71 ppm (Meretoja et al., 1977; Fleig and Thiess, 1978; Andersson et al., 1980; Camurri et al., 1983; Hogstedt et al., 1979). Chromosomal aberrations were not increased in workers exposed to 30-<700, 6-58 or <1 ppm (Watanabe et al., 1981; Thiess et al., 1980; Fleig and Thiess, 1978).

The controlled experiments with humans and the animal toxicity studies indicate that neurophysiological effects are sensitive indicators of styrene toxicity. A number of occupational studies have reported effects of styrene on neurological function (see Table VI-1). Increased reaction time, reported in a controlled experiment with humans (Gamberale and Hultengren, 1974), has been recorded in workers occupationally exposed to styrene. Gotell et al. (1972) found significantly (p<0.01) longer mean simple reaction times measured both before and after work in six subjects exposed to >150 ppm TWA styrene for $\leq$12 years compared with six age-matched controls from a motor workshop. No significant difference in reaction time was observed between 11 workers exposed to <150 ppm for $\leq$12 years and 11 age-matched controls. Neither exposure group differed significantly from controls with respect to reflexes, vibration sensitivity or Romberg test (Gotell et al., 1972). WHO (1982) summarized two studies for which the original papers could not be obtained. Gamberale et al. (1975) found longer and more irregular reaction times in 106 workers exposed to 13-101 ppm styrene for a duration of $\leq$11 years (mean 2.7 years) than in an uncharacterized control group. These effects were still evident after a nights' rest. In the second paper summarized by WHO (1982), Kjellberg et al. (1979) found that seven workers exposed to a mean concentration of 9 ppm styrene for an average of 10 years had longer reaction times after work than before, whereas afternoon reaction times in an uncharacterized control group were shorter than morning times. Performance was unimpaired on unspecified tests of sensory and motor functions or an addition task. Cherry et al. (1980) found the mean reaction time was slower in 27 workers exposed to a mean TWA of 92 ppm styrene on a Monday morning after a weekend with no exposure than in 27 unexposed workers from the same factory. However, reaction times of exposed workers were improved in the afternoon following styrene exposure; the afternoon reaction times were similar to controls. A subgroup of workers with high blood styrene concentrations ($\geq$5.5 μmol/ℓ) showed little improvement in reaction time between morning and afternoon. Other tests (vigilance, digit-symbol substitution, forward and backward digit-span) revealed no significant alterations in performance of styrene-exposed workers. Rosen et al. (1978) found some indication of sensory neuropathy in styrene-exposed workers. Mean duration of SAPs at the wrist was significantly ($p<0.005$) longer for 12 workers exposed to 125 ppm TWA styrene (1-12 years) and 10 workers exposed to <5 ppm TWA styrene compared with 6 controls. Amplitude of the SAPs was significantly ($p<0.05$) lower in 10 workers exposed to 47 ppm TWA styrene (2-14 years) compared with the 6 controls. Motor conduction velocity was not affected.

A cohort of 98 Finnish male laminating workers has been intensively studied. Lindstrom et al. (1976) performed 10 tests measuring intelligence, memory, vigilance, personality, visuomotor speed and accuracy, and psychomotor performance. The 98 workers exposed to styrene for ≤14 years (mean 4.9) with urinary mandelic acid levels ranging between 7 and 4715 mg/ℓ had significantly ($p<0.05$) poorer visuomotor accuracy and longer latency time in the Rorschach test than 43 concrete enforcement workers with similar age distribution and educational level. Workers with higher levels of mandelic acid (1762-4715 mg/ℓ) had significantly ($p<0.005$) poorer visuomotor accuracy and psychomotor performance than low-exposure workers. The psychological exams were performed ≥20 hours after the last exposure so the authors (Lindstrom et al., 1976) considered the results representative of cumulative effects of chronic exposure. Harkonen et al. (1978) analyzed the exposure-response relationships of styrene exposure and neurological function based on the results from the cohort of Finnish workers. The workers were classified into groups with mean mandelic acid concentrations <800, 800-1199, 1200-1599, 1600-1999 and >2000 mg/ℓ. Chi-square analysis of the results of the Symmetry Drawing test indicated that the incidence of reduced visuomotor accuracy increased significantly ($p<0.05$) when the mean mandelic acid concentration exceeded 800 mg/ℓ. A more pronounced decrease in visuomotor and psychomotor performance was observed at >1200 mg/ℓ.

Harkonen et al. (1978) also analyzed the proportion of normal and abnormal EEGs obtained from 96 of the 98 exposed workers. A marked increase in the proportion of abnormal EEGs occurred when the mandelic acid concentration was ≥700 mg/ℓ. These mandelic acid concentrations were converted to estimated 8-hour TWA styrene concentrations based on a regression equation previously developed by the research group Engstrom et al. (1976). The threshold for deterioration in psychological function was estimated as 36 ppm TWA styrene, while the threshold for an increased incidence of abnormal EEGs was ~31 ppm TWA, and the marked decrease in visuomotor and psychomotor performance was observed at ~55 ppm TWA. Based on the thresholds determined by Harkonen et al. (1978), it appears that an 8-hour TWA of ~30 ppm would represent a NOAEL when the endpoint of interest is psychological or neurophysiological function. The other studies are somewhat supportive of this NOAEL. The results of Gotell et al. (1972) suggest a NOAEL of ~150 ppm, but Gamberale et al. (1975) noted an adverse effect on reaction time in workers exposed to 13-101 ppm and Kjellberg et al. (1979) found altered reaction times in workers exposed to a mean concentration of only 9 ppm. Rosen et al. (1978) found indications of mild sensory neuropathy at exposure levels of <5, 47 or 125 ppm.

An assessment of the health effects of styrene should consider the effects of styrene on the fetuses of pregnant women. There are only three available studies that discuss possible reproductive effects of styrene in humans. Hemminki et al. (1980a) found significant ($p<0.01$) increases in the rate and ratio of spontaneous abortions in Finnish styrene industry workers compared with the rate among all Finnish women, but the sample size for the styrene workers was small (6 spontaneous abortions), the exposure levels were not quantified and the exposed workers were not characterized.

Holmberg (1977) reported that mothers of 2/43 children born with CNS defects in Finland during a 9-month period had occupational exposure to styrene. The expected number of births in styrene-exposed workers during that period was 12. A rate of 2/12 infants with CNS defects was compared with the expected rate of CNS defects of 0.5/1000. The low number of births precluded statistical analysis of the results and no quantitative exposure data were available. The two reports suggest that the fetus may be more sensitive than the adult to the toxic effects of styrene. Spontaneous abortion rates did not differ between 67 controls and 67 lamination workers, with styrene exposure estimated (from a previous study) as 66 ppm (Harkonen and Holmberg, 1982).

The teratogenic or fetotoxic potential of styrene can be further evaluated from the results of animal studies. Murray et al. (1976, 1978a) found no teratogenic or fetotoxic effects when 29-30 rats were dosed with 180 or 300 mg/kg/day on days 6-15 of gestation (doses of 90 and 150 mg/kg were administered twice daily). A decrease in maternal body weight and food consumption was seen during the first 24 hours of the experimental period, but maternal body weight gain did not differ significantly from controls at subsequent measurement times. Maternal liver weights on day 21 of gestation were not significantly different from controls. An additional group of seven pregnant rats were dosed with 300 mg/kg/day of styrene on days 6-15 of gestation. Gross necropsy on day 16 of gestation revealed no signs of toxicity with the possible exception of focal gastric ulceration suggested by the presence of pinpoint darkened areas in the stomach of 4/7 rats. This effect was not observed in six controls (Murray et al., 1976).

In a 3-generation reproduction study by Beliles et al. (1985), styrene was administered to Sprague-Dawley rats in the drinking water at 0, 7.7 and 14 mg/kg in males and 0, 12 and 21 mg/kg in females. No treatment-related effects were observed for fertility, litter size, pup viability, pup survival, sex ratio, pup body weight, weanling liver and kidney weight, and marrow cytogenetics.

In an inhalation study, no fetotoxic or teratogenic effects were observed in rats exposed to 300 or 600 ppm, 7 hours/day on days 6-15 of gestation (Murray et al., 1978a,b). There was a significantly ($p<0.05$) smaller crown-rump length in the fetuses of the 300 ppm group (43.8 mm) compared with concurrent controls (44.8 mm). This effect was not observed at 600 ppm and the decrease would not be significant compared with the controls for the 600 ppm group. Maternal body weight gain on days 6-9 (but not days 10-15, 16-20) was significantly ($p<0.05$) reduced at both exposures. Rabbits exposed to 300 or 600 ppm, 7 hours/day on days 6-16 of gestation exhibited no signs of maternotoxicity, fetotoxicity or teratogenicity (Murray et al., 1978a,b). Kankaanpaa et al. (1980) found a significant ($p<0.001$) increase in the number of dead or resorbed fetuses in hamsters exposed to 1000 ppm, 6 hours/day on days 6-18 of gestation. No fetotoxic effects were observed at exposure concentrations of 300, 500 or 750 ppm and no teratogenic effects were observed at any exposure level.

In contrast to the above results for rats, rabbits and hamsters, Kankaanpaa et al. (1980) found an increase ($p<0.1$) in the number of dead or resorbed fetuses (26.9%, compared with 18.2% for controls) in mice at exposure levels of 250 ppm, 6 hours/day on days 6-16 of gestation. In preliminary experiments, a similar exposure schedule using 500 ppm styrene resulted in maternal deaths in 2/6 of the mice and a fetal death rate of 47% in the surviving mice. At 750 ppm, 3/5 mice died and the fetal death rate was 95% among the offspring of the 2 survivors.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day HA. The 1-day HA can be derived from the results of a controlled styrene inhalation exposure study using nine healthy human male volunteers (Stewart et al., 1968). No subjective or objective signs of toxicity were noted following 1- and 2-hour exposures to 51 ppm (217 mg/m^3) or 117 ppm (499 mg/m^3) styrene. To simulate a workday, six subjects were exposed to 99 ppm (422 mg/m^3) styrene vapor for two 3.5-hour periods separated by a 30-minute period without exposure. Flannagan Coordination and Crawford Manual Dexterity tests for these subjects were unchanged from pre-exposure scores. Three of the six subjects did report intermittent difficulty performing the modified Romberg test. However, during the eight times this test was performed two of the subjects reported difficulty only one time and the third two times. In contrast, exposure to 376 ppm (1602 mg/m^3) styrene vapor for 1 hour resulted in abnormal neurological findings and complaints of nausea and inebriation. The results of urinalysis, hematology and blood chemistry studies, which were conducted 16 and 72 hours after all exposures, were normal and unchanged from pre-exposure values. The results of another study using human volunteers exposed to similar styrene levels indicate the mean pulmonary styrene uptake is 64% of the inspired amount (Odkvist et al., 1982). Using a NOAEL of 99 ppm (422 mg/m^3) from a 7-hour exposure, the 1-day HA would be derived as follows:

$$\text{TAD} = \frac{(422\ \text{mg/m}^3)\ (20\ \text{m}^3\text{/day})\ (7\ \text{hours/24 hours})\ (0.64)}{70\ \text{kg}} = 22.5\ \text{mg/kg/day}$$

where:

TAD = total absorbed dose

422 mg/m^3 = NOAEL, converted from 99 ppm exposures in humans (Stewart et al., 1968)

7 hours/24 hours = duration of exposure/day

20 m^3/day = assumed ventilation volume for 70 kg adult

0.64 = estimated ratio of absorbed dose (Odkvist et al., 1982)

70 kg = weight of exposed individual (adult)

$$\text{1-Day HA} = \frac{22.5\ \text{mg/kg/day} \times 10\ \text{kg}}{10 \times 1\ \ell\text{/day}} = 22.5\ \text{mg/}\ell$$

where:

22.5 mg/kg/day = total absorbed dose (TAD)

10 kg = weight of protected individual (child)

10 = uncertainty factor, appropriate for use with human NOAEL, to accomodate for intraspecies sensitivity

1 ℓ/day = assumed volume of water consumed by a 10 kg child

The proposed 1-day HA is 22.5 mg/ℓ for a 10 kg child.

Derivation of 10-Day HA. No data were identified in the available literature that would be suitable for the derivation of a 10-day HA. Therefore, the longer-term HA of 20 mg/ℓ for a 10 kg child is suggested as a conservative estimate of exposure.

Derivation of Longer-Term HA. Suitable data are available for the derivation of longer-term HAs. Quast et al. (1979) reported minimal toxicologic changes when four beagle dogs/sex/dose group were gavaged with 0, 200, 400 or 600 mg/kg bw/day styrene in peanut oil for 561 days. Results of this study were also summarized in an abstract (Quast et al., 1978). No dose-related alterations in body or organ weights, urinalysis or clinical chemistry determinations were detected. No dose-related effects were detected in dogs administered 200 mg/kg/day styrene. Effects at 400 or 600 mg/kg/day were increased numbers of Heinz bodies in the red blood cells, decreased packed cell volume and sporadic decreases in hemoglobin and red blood cell counts; increased iron deposits and elevated numbers of Heinz bodies were found in the livers. At 600 mg/kg/day hepatocellular inclusions were observed. Similarly, no overt signs of toxicity (i.e. organ or body weight changes) were observed in rats exposed orally to styrene at 200 or 400 mg/kg/day, 6 days/week for 100 days (Srivastava et al., 1982). Focal liver necrosis and significantly elevated serum transaminase activities were observed at the higher dose only. Taking 200 mg/kg/day as the NOAEL (Quast et al., 1979), the longer-term HAs can be derived as follows:

$$\text{Longer-term HA (child)} = \frac{200 \text{ mg/kg/day} \times 10 \text{ kg}}{100 \times 1 \text{ ℓ}} = 20 \text{ mg/ℓ}$$

where:

200 mg/kg/day = NOAEL for oral exposure in dogs (Quast et al., 1979)

10 kg = weight of protected individual (child)

100 = uncertainty factor appropriate for use with a NOAEL from animal data and to protect sensitive members of the human population

1 ℓ/day = assumed volume of water consumed by a 10 kg child

$$\text{Longer-term HA (adult)} = \frac{200 \text{ mg/kg/day} \times 70 \text{ kg}}{100 \times 2 \text{ ℓ}} = 70 \text{ mg/ℓ}$$

where:

200 mg/kg/day = NOAEL for oral exposure in dogs (Quast et al., 1979)

70 kg = weight of protected individual (adult)

100 = uncertainty factor appropriate for use with a NOAEL from animal data and to protect sensitive members of the human population

2 ℓ/day = assumed volume of water consumed by a 70 kg adult

It is recommended that 20 mg/ℓ and 70 mg/ℓ be used as the longer-term HA for a 10 kg child and a 70 kg adult, respectively.

Assessment of Lifetime Exposure and Derivation of a DWEL. Quast et al. (1979) is also adequate for use in deriving the lifetime DWEL. The study in beagle dogs reported a NOAEL of 200 mg/kg/day following exposure for 561 days. Similar findings were observed in rats administered doses of 66.7, 133, 400 or 667 mg/kg/day styrene for 6 months (Wolf et al., 1956). At 400 or 667 mg/kg/day, decreased growth weights and increased liver and kidney weights were observed, without hematologic or histopathologic effects. At the two lower dose levels, no effects were noted on body weight, organ weight or pathology. Using Quast et al. (1979) a DWEL for styrene is derived as follows:

Step 1 - RfD Derivation

$$\text{RfD} = \frac{200 \text{ mg/kg/day}}{1000} = 0.2 \text{ mg/kg/day}$$

where:

200 mg/kg/day = NOAEL for oral exposure in dogs (Quast et al., 1979)

1000 = uncertainty factor appropriate for use with a NOAEL from animal data, less than lifetime exposure and to protect sensitive members of the human population.

Step 2 - DWEL Derivation

$$\text{DWEL} = \frac{0.2\ \text{mg/kg/day} \times 70\ \text{kg}}{2\ \text{ℓ/day}} = 7.0\ \text{mg/ℓ}$$

where:

0.2 mg/kg/day = RfD

70 kg = assumed weight of protected individual (adult)

2 ℓ/day = assumed volume of water consumed by a 70 kg adult

The recommended lifetime DWEL for a 70 kg adult is 7.0 mg/ℓ styrene.

A summary of the data used to calculate the HAs and the lifetime DWEL is provided in Table VIII-1. The values derived for the HAs and DWEL represent estimates of the concentration of styrene in drinking water that will not cause adverse effects after 1-day, 10-day, longer-term or lifetime exposures.

Carcinogenic Effects

Three animal bioassays provide evidence of styrene carcinogenicity. Styrene was administered by gavage in two of the studies and in the third by inhalation. In an IARC-sponsored study (Ponomarkov and Tomatis, 1978), pregnant mice (O_{20} and C57Bl strains) and rats (BDIV) received single doses of styrene by gavage at 17 days of gestation. The O_{20} mice received 1350 mg/kg bw, the C57Bl mice received 300 mg/kg bw and the BDIV rats received 1350 mg/kg bw. Beginning at weaning, all progeny received the same dose levels as the mothers administered once weekly for life. In O_{20} mice there was excessive mortality in offspring during the preweaning period; mortality in progeny from treated and control mothers was 43 and 22%, respectively. This high mortality rate persisted even though treatment was

TABLE VIII-1

Summary of Data Used to Derive the HAs and DWEL

Criteria	Animal Dose	Duration	Effect	Value of HA or DWEL		Reference
				Adult	Child	
1-Day HA	22.5 mg/kg (human NOAEL)	7 hours	higher doses cause CNS toxicity		22.5 mg/ℓ	Stewart et al., 1968
10-Day HA	200 mg/kg (dog NOAEL)	561 days	higher doses cause hepatotoxicity and hematological effects		20 mg/ℓ*	Quast et al., 1979
Longer-term HA	200 mg/kg (dog NOAEL)	561 days	higher doses cause hepatotoxicity and hematological effects	70 mg/ℓ	20 mg/ℓ	Quast et al., 1979
Lifetime DWEL	200 mg/kg (dog NOAEL)	561 days	higher doses cause hepatotoxicity and hematological effects	7.0 mg/ℓ	NA	Quast et al., 1979

*The 10-day HA is a conservative estimate of exposure derived from the longer-term HA.

NA = Not applicable

terminated at 16 weeks because of toxicity. Characteristically, O_{20} mice have a high spontaneous rate of lung adenomas and carcinomas; in this study, the concurrent incidence of males and females was 42 and 67%, respectively, with an average age at tumor onset of 84 and 85 weeks. Male and female styrene-treated mice, on the other hand, had lung tumor incidences of 89 and 100%, respectively, with a corresponding earlier age of tumor onset of 49 and 58 weeks. The excess in tumor incidence in styrene-treated groups was statistically significant for both sexes ($p<0.02$ for males, $p<0.001$ for females). The high spontaneous background rate of lung tumors in O_{20} mice raises a question as to how to interpret this increase in tumor incidence. One accepted approach to this problem of assessing the carcinogenicity when high background tumor rates are involved has been employed by Stoner and Shimkin (1982) and Shimkin and Stoner (1975), who have analyzed tumor data on the basis of the number of tumors per animal rather than the number of animals with tumors. Analysis of the O_{20} mouse data on the basis of number of tumors per animal gives virtually the same statistical significance as the analysis based on number of animals with tumors. This is because very few animals had more than one tumor, so it makes little difference whether tumors or animals are counted. The observed significant increase in tumor incidence cannot be dismissed and is thought to be of a highly suggestive nature given the high statistical significance of the response and the reduced latency period for tumor induction.

In the other animal species used (Ponomarkov and Tomatis, 1978), no significant styrene-related effects were reported by the authors. In C57Bl mice an increase in liver carcinomas was observed in treated animals, however (3/24). Although this increase is not statistically significant when compared with male vehicle controls (0/12), it is statistically significant (p=0.022) when compared with male untreated controls (0/47) and vehicle controls combined. Pooling both control groups is considered to be proper because there is no difference between their tumor responses. No significant tumor-related effects were observed in BDIV rats. As the dosing in these assays (once per week) was not continuous, the sensitivity of these assays is thought to be less than optimum.

In the NCI (1979) study, male and female B6C3F1 mice and Fischer 344 rats were treated by gavage 5 days/week for 78 weeks, or in the case of the low-dose rat groups, 103 weeks. This study was terminated at 91 weeks (mice) and 104-105 weeks (rats). Increased tumor incidence was observed only in male B6C3F1 mice in this study. A dose-related increase in alveolar/bronchiolar adenomas and carcinomas was observed, with the low-dose (150 mg/kg bw) and high-dose (300 mg/kg bw) groups showing, respectively, tumor incidence of 14% (6/44) and 21% (9/43; p=0.024 by Fisher exact test) compared with a 0% (0/20) incidence in the vehicle control group. The dose-response trend for combined adenomas and carcinomas was also significant (p=0.023). Despite its positive aspects (a significant dose-response trend and a significantly higher incidence of tumors at the high dose), this study was considered by NCI to provide only "suggestive" evidence for carcinogenicity in the male mouse. The validity of the lung tumor response was questioned because the concurrent vehicle control incidence of 0/20 was considered to be abnormally low inasmuch as the historical nonvehicle control incidence in this laboratory was 12% (32/271), and on two occasions has been as high as 20% (4/20). It should be noted, however, that the observed 0/20 vehicle concurrent control incidence in this study is not unprecedented inasmuch as the historical rate for vehicle controls in this laboratory was 0/40.

In a CMA-sponsored inhalation study (Jersey et al., 1978), male and female Sprague-Dawley rats were exposed to styrene vapor at 0, 600 or 1200 ppm for 6 hours/day, 5 days/week for 18-20 months. The highest dose was reduced to 1000 ppm after 2 months because of decreased weight gain in males. The study was terminated at the end of 2 years. An increased incidence (8%; 7/85) of mammary adenocarcinomas was reported in the low-dose female group only; no tumors were reported in the high-dose group. The control incidence for this tissue site was 1.2% (1/85). Although statistically significant (p=0.03, Fisher's exact test), this response was questioned by the authors because no increase in tumors was observed in the high-dose group and the historical control rate for this tumor site in the same laboratory during the 5 years before this study ranged from 0-9.3% with a mean of 5.8%, a value well above the concurrent control rate.

An increased incidence of leukemia/lymphosarcomas, only in females, was also observed in the Jersey et al. (1978) study, with both dose groups showing identical incidences of 7% (6/85) compared with a concurrent control incidence of 1.2% (1/85). The mean historical control rate for this group of neoplasms is 1.06% (range, 0-2.6%) in this laboratory. The absence of a dose-related increase in tumor incidence in this study at the high dose may be related, in part, to the observation that metabolic clearance of styrene becomes saturated at levels from 200-600 ppm in the rat (Young et al., 1979). The increased incidence of these neoplasms was considered by the authors of the study not to be statistically significant (p=0.06) when compared with concurrent controls, although a trend was noted. With a P value of 0.06, the response may be better characterized as having a borderline significance. The increase was found to be highly significant (p=0.003), however, when compared with the historical controls for this tumor site. The study results for the males were confounded by "excessive" nontreatment-related mortality, which occurred principally in the control and high-dose groups. In view of the various uncertainties surrounding the data, this study, although giving a positive response, was considered by its authors to provide only suggestive evidence for carcinogenicity. It should be emphasized, however, that the authors of the study have tended to downgrade the statistically significant increase in mammary adenocarcinomas on the basis of historical control data (because the mean historical control incidence was well above that of the concurrent controls and high-dose groups) while at the same time they have not emphasized that the increased incidence of leukemia/lymphosarcoma, although borderline relative to concurrent controls, was highly significant when compared with historical controls for this tumor.

In a carcinogenicity bioassay by Conti et al. (1988) Sprague-Dawley rats were treated with styrene by inhalation 4 hours/day, 5 days/week for 52 weeks at 0, 25, 50, 100, 200 and 300 ppm. There was a higher incidence of total (benign and malignant mammary tumors) and malignant mammary tumors in the females of all the groups exposed to styrene when compared with controls. The incidence of malignant mammary tumors was treatment-related and statistically significant. Styrene was administered by ingestion, stomach tube (0, 50 and 250 mg/kg in olive oil, once daily, 4-5 days/week for 52 weeks), by intraperitoneal injection (0 and 50 mg in olive oil 4 times at 2-month intervals) and by subcutaneous injection (0 and 50 mg in olive oil, once). No other significant carcinogenic effects were observed in the animals treated by inhalation. No significant treatment-related increases in tumor incidence were observed in animals treated by either ingestion or injection. It should be noted that female rats exposed by ingestion to 50 mg/kg styrene had increased incidence in total mammary tumors (benign and malignant); however, due to high mortality at the 250 mg/kg dose no increase in tumor incidence was reported.

Because of the limitations cited above for each of the animal bioassays, neither study, when considered separately, is strong enough to satisfy the criteria outlined in the Cancer Risk Assessment Guidelines for "sufficient evidence". There were, nevertheless, increased tumor incidences in each study and the observed increases for some tumor sites were statistically significant when compared with concurrent controls. In addition, these positive responses involved the following: 1) multiple strains, species and sexes; 2) a dose-response effect (NCI, 1979); 3) a tumor response in treated groups, which occurred at a significantly earlier age than in control groups (Ponomarkov and Tomatis, 1978); and 4) tumor induction at the same site (lung) following styrene administration by the same route (gavage) in two separate studies (Ponomarkov and Tomatis, 1978; NCI, 1979). Therefore, when the data from these studies are considered collectively, the criteria for "sufficient evidence" of carcinogenicity as outlined in the guidelines are satisfied (U.S. EPA, 1986). The judgment of "sufficent evidence" from the bioassays alone is regarded as marginal because of weaknesses in each of the bioassays, which have the influence of providing less than clear evidence.

Strong additional supporting evidence for the carcinogenicity of styrene comes from metabolic and genotoxicity studies. The major route for styrene metabolism in humans as well as experimental animals is through styrene-7,8-

oxide. Support for this consensus comes from extensive metabolism studies in experimental animals and humans (see Chapter III, Metabolism Section). Additional support comes from direct animal evidence showing that the epoxide can accumulate in measurable quantities in various mouse tissues (brain, liver, blood, subcutaneous adipose tissue and lung) following styrene injection (Nordqvist et al., 1985; Löf et al., 1984). Direct human evidence comes from a recent report (Löf et al., 1986) demonstrating that the epoxide can be measured (although at the detection limit in most cases) in the blood of workers exposed to styrene in glass-fiber reinforced plastics factories. All available evidence indicates that styrene-7,8-oxide is the initial product in the metabolism of >90% of the styrene catabolized by both experimental animals and humans and that this epoxide, although a highly reactive intermediate, is stable for a sufficient period of time to be detectable in tissues. It is also significant that styrene has been shown in humans to accumulate in subcutaneous adipose tissue with a half-life of 2-5 days (Engström et al., 1978a,b) and thus, may be available for metabolism to the epoxide over prolonged periods of time in the absence of repeated exposure.

In three animals studies (Maltoni et al., 1979; Lijinsky, 1986; Ponomarkov et al., 1984), styrene-7,8-oxide was shown to be a potent animal carcinogen. The epoxide, when given by gavage, was shown to induce a high incidence of forestomach carcinomas in rats and mice (both sexes). Maltoni et al. (1979) administered styrene oxide at levels of 4.12 or 8.25 mg/kg daily by olive oil gavage to male and female Sprague-Dawley rats. Exposure commenced when the rats were 13 weeks old and continued for 52 weeks. The study was terminated when the rats were 148 weeks old. Statistically significant increased incidences of forestomach epithelial squamocellular carcinomas were observed in both exposed groups of each sex with statistically significant dose-related trends. Details of this study are given in the carcinogenicity section of Chapter V.

Lijinsky (1986) administered styrene oxide by corn oil gavage to male and female F344 rats and B6C3F1 mice. Statistically significant increased incidences of forestomach carcinomas or papillomas were observed in both exposed groups of each sex and species, with statistically significant dose-related trends in the rats. Details of this study are given in the carcinogenicity section of Chapter V. Styrene oxide (100-150 mg/kg bw) produced statistically significant benign and malignant forestomach tumors in both sexes of BDIV rats after gavage administration for 96 weeks (Ponomarkov et al., 1984). Styrene oxide (0, 50 and 250 mg/kg) produced a dose-related increase in total and malignant tumors in the forestomach when treated by ingestion (stomach tube) in olive oil, once daily, 4-5 days/week for 52 weeks (Conti et al., 1988). Two other tumor types (lung and nervous system), which are rare in this strain, were noted; however, their incidences did not reach statistically significant levels. Details of this study are given in the Carcinogenicity Section of Chapter V.

The animal bioassay data on styrene-7,8-oxide were evaluated by IARC (1985) and considered to provide sufficient evidence for carcinogenicity in experimental animals; no human data were available. On the basis of sufficient animal evidence and the absence of human data, IARC considered styrene-7,8-oxide to present a carcinogenic risk to humans (IARC, 1985). According to the revised IARC evaluation program (IARC, 1987) the oxide would be classified as either a 2B possible human carcinogen or, if the metabolism/genotoxicity data were considered as supporting evidence, as a 2A probable human carcinogen. These two classifications are equivalent, respectively, to EPA classifications B2 and B1/B2, both of which are in the probable human carcinogen category.

The ability of the epoxide to cause carcinomas at the site of tissue contact demonstrated in the previous three studies is of particular interest in view of reports that this highly reactive intermediate has been detected in ambient air of plastics manufacturing plants (Fjelstad et al., 1979). The lung may be of particular concern in this respect because this tissue has been shown in some species (mice and rabbits; human lung was not investigated) to be particularly inefficient in metabolizing the epoxide to its glycol metabolite because of an unusually low epoxide hydrolase specific activity relative to monooxygenase levels (Cantoni et al., 1978). The lung, therefore, may be particularly vulnerable to exposure by both styrene and the epoxide because, although the tissue readily metabolizes styrene to the epoxide, it is relatively inefficient in removing this potent mutagen and carcinogen by hydrolase activity. In considering this putative vulnerability of mouse lung to styrene and its epoxide, it is interesting that in both styrene bioassays carried out in mice (Ponomarkov and Tomatis, 1978; NCI, 1979), increased incidences of lung tumors were observed, even though the route of administration was by gavage.

Styrene has been tested for mutagenicity in a variety of systems, including bacteria, yeast, cultured mammalian cells, cultured human lymphocytes, fruit flies and *in vivo* mammalian assays (see Mutagenicity Section in Chapter V). Although there are conflicting reports on the mutagenicity of styrene in bacterial test systems, a considerable body of *in vivo* and *in vitro* evidence has been presented in support of styrene as a genotoxic agent.

It is generally assumed that the genotoxic effects of styrene are mediated by the epoxide metabolite. Styrene has been shown to cause recessive lethal mutations in *Drosophila* and gene conversions in yeast (Loprieno et al., 1976; Donner et al., 1979). In mammalian cells in culture, styrene has been reported to cause mutations at the HGPRT locus in V79 cells (Beije and Jenssen, 1982), chromosomal aberrations in CHL cells and human lymphocytes (Matsuoka et al., 1979; Ishidate and Yoshikawa, 1980; Linnainmaa et al., 1978a,b), and sister chromatid exchange (SCE) in CHO cells and human lymphocytes (Linnainmaa et al., 1978b; Norppa et al., 1980a). Micronuclei have also been reported to be induced by styrene in cultured human lymphocytes (Vainio et al., 1984). The induction of mutations, chromosomal aberrations and SCEs by styrene in Chinese hamster cells required a metabolic activation system (cited in Vainio et al., 1984). In human lymphocyte cultures, on the other hand, prior metabolic activation was not required for the induction of chromosomal aberrations, micronuclei and SCEs (Linnainmaa et al., 1978b; Norppa et al., 1980a). The genotoxic effects of styrene on lymphocytes have been studied in whole blood cultures, however, and it has been demonstrated that both lymphocytes and erythrocytes have the ability to metabolize styrene to styrene-7,8-oxide (Belvedere and Tursi, 1981). The level of SCEs in cultured human lymphocytes, in fact, has been shown to be a function of the erythrocyte concentration (Norppa et al., 1983). This metabolism is not dependent on NADPH and, therefore, probably is not mediated by cytochrome P-450; current evidence suggests that erythrocyte oxyhemoglobin is responsible for this metabolism (Tursi et al., 1983).

Genotoxic effects of styrene are not confined to *in vitro* studies. Styrene has been shown, *in vivo*, to cause chromosomal aberrations in rat bone marrow cells, micronuclei and SCE in mouse marrow, and SCE in mouse alveolar macrophages and regenerating liver cells (Conner et al., 1982; Norppa, 1981; Meretoja et al., 1978a). Elevated levels of single-strand breaks (SSBs) in DNA have been reported in various mouse organs (kidney, liver, lung, testis, brain) following intraperitoneal injection of styrene. Once elevated, the levels of SSBs persisted in lung, liver and testis for a period in excess of 24 hours (Walles and Orsen, 1983).

A number of studies have shown increased incidences of chromosomal aberrations and SCEs in human peripheral lymphocytes following occupational exposure to styrene (Norppa et al., 1980a; Meretoja et al., 1978a; Fabry et al., 1978). One study of particular interest also showed that lymphocytes of styrene-exposed workers were more susceptible to sustaining DNA damage when exposed to genotoxic agents (*in vitro*) than were lymphocytes from unexposed workers. It was concluded that styrene exposure may also "sensitize" lymphocytes to DNA damage by other environmental mutagens (Pero et al., 1982).

Finally, a recent study (Nordquist et al., 1985) has provided the first evidence that styrene forms protein and DNA adducts. Covalent binding of both 7-[^{14}C] styrene and its oxide was demonstrated in mouse plasma protein, hemoglobin and DNA from liver, lung and brain following intraperitoneal injection. The DNA adduct levels (at Gua-N-7) in these tissues was comparable to that produced by ethylene oxide, a clearly-demonstrated mutagen.

All of the above genotoxicity effects of styrene have also been shown to be caused by styrene-7,8-oxide, with the notable distinction that in cases where prior metabolic activation is required to obtain an effect with styrene, such as in Chinese hamster cells, no prior activation is required for the epoxide (cited in Vainio et al., 1984). This evidence, along with recent data showing that styrene and its epoxide form DNA adducts and that the epoxide has been detected in human blood, provides strong additional supporting evidence for the human carcinogenic potential of styrene.

The carcinogenic potential of styrene has not been further clarified by studies of humans occupationally exposed to styrene. Multiple chemical exposures of the study populations or small cohort sizes limit the conclusions that can be drawn from these studies. Reports by McMichael et al.(1976), Block (1976) and Lemen and Young (1976) indicated there may be an elevated risk of hematopoietic and lymphatic cancer for workers in the styrene-butadiene rubber industry. In a recent retrospective cohort mortality study conducted by NIOSH, Meinhardt et al. (1982) reported that the incidence of lymphatic and hematopoietic neoplasms was not significantly elevated in workers from two styrene-butadiene rubber plants. A subgroup of workers with unquantified higher styrene exposure (employed before manufacturing process changes) had an elevated standardized mortality ratio for leukemia and aleukemia that was not a statistically significant elevation using the two-sided test customarily employed by NIOSH, but this elevation was significant (p<0.05) using a one-sided test (Meinhardt et al., 1982). Excesses of leukemia in styrene-exposed workers in other industries have been reported by Ott et al. (1980) and Nicholson et al. (1978), although the increase was not statistically significant in the study by Nicholson et al. (1978). The incidence of cancer deaths was not elevated in a study of styrene-polystyrene workers conducted by Frentzel-Beyme et al. (1978). An elevated risk of digestive system cancers in styrene-polystyrene workers was reported in an abstract (Liebling et al., 1982). Hodgson and Jones (1985) found a significant elevation of lymphoma deaths in workers engaged in styrene production, polimerization and processing. However, the number of lymphoma deaths was small (3) and workers were exposed to other chemicals in addition to styrene.

Quantification of Carcinogenic Effects

The results of three chronic animal bioassays (Jersey et al., 1978; NCI, 1979; Ponomarkov and Tomatis, 1978; Conti et al., 1988) collectively provide sufficient animal evidence for the carcinogenic activity of styrene. Strong supporting evidence is provided by metabolic and genotoxicity studies, some of which have been published recently. When the animal bioassay data are considered collectively along with the metabolism/genotoxicity data. The guidelines provide several avenues for reaching a "sufficient" level of animal evidence. In this analysis, the bioassay data alone were considered to be strong enough for at least a "marginal" call of sufficient animal evidence. The weight-of-evidence classification of styrene is currently undergoing Agency review. This document will be revised when this review has been completed.

The classification of marginally-sufficient animal evidence comes from animal bioassays showing statistically significant increased tumor incidences in the B6C3F1 male mouse (alveolar/bronchiolar adenomas and carcinomas by multiple dose gavage) (NCI, 1979), in male and female 0_{20} mice (lung adenomas and carcinomas by dose gavage), in male C57Bl mice (liver carcinomas by gavage) (Ponomarkov and Tomatis, 1978), in female Sprague-

Dawley rats (leukemia/lymphosarcoma, by inhalation) (Jersey et al., 1978) and in female Sprague-Dawley rats (mammary tumor, by inhalation) (Conti et al., 1988).

The guidelines encourage the use of additional considerations to support or limit the strength of the bioassay evidence. In the case of styrene, the evidence for genotoxicity in short-term animal test systems along with recent data showing that styrene and its epoxide metabolite form DNA adducts and that the epoxide has been detected in humans exposed to styrene is very supportive of a carcinogenic potential. Equally important is the fact that the epoxide metabolite has been tested in rodents and found to be clearly carcinogenic in this bioassay.

Direct evidence that inhalation of styrene causes cancer in laboratory animals comes from the study by Jersey et al. (1978). Groups of male or female Sprague-Dawley rats were exposed for 6 hours/day, 5 days/week to styrene vapor at levels of 600 or 1200 ppm. The high dose was reduced to 1000 ppm after 2 months. Exposure was terminated when half the exposed animals died, which was at 18.3 months for male rats and 20.7 months for female rats. The study was terminated after 24 months. Statistically significant increases of leukemia and lymphosarcomas combined were observed in females exposed to 600 and 1000 ppm and in males exposed to 600 ppm. Details of these studies are given in Tables VIII-2 and VIII-3.

To estimate the cancer potency the exposures are prorated over the animals' lifetime. Although this approach can be problematical when the exposure is intense but of short duration, in the absence of evidence to the

TABLE VIII-2

Summary of Tumor Incidence in Female Rats

Substance:	Styrene		
Reference:	Jersey et al., 1978		
Sex, Strain, Species:	Female, Sprague-Dawley (Spartan substrain) rat		
Exposure route, Vehicle:	Inhalation of vapor		
Tumor site, Type:	Leukemia and lymphosarcoma		
Nominal dose:	0	600	1000 ppm (30 hours/week)
Average daily dose:	0	89	151 ppm (as if continuous)
	0	235	399 mg/kg/day
Equivalent human dose:	0	41	70 mg/kg/day (surface-area adjusted)
Tumor incidence:	1/85	6/85	6/85
Tumor percentage:	1%	7%	7%
Statistical significance:	--	0.06	0.06
Trend significance:	<0.05	linearity OK	
Animal weight:	0.384 kg (control, at end of exposure period)		
Exposure period:	20.7 months		
Study length:	24 months		
Animal lifespan:	24 months		
Potency (q_1^*):	2E-03 (mg/kg/day)		

TABLE VIII-3

Summary of Tumor Incidence in Male Rats

Substance:	Styrene		
Reference:	Jersey et al., 1978		
Sex, Strain, Species:	Male, Sprague-Dawley (Spartan substrain) rat		
Exposure route, Vehicle:	Inhalation of vapor		
Tumor site, Type:	Leukemia and lymphosarcoma		
Nominal dose:	0	600	1000 ppm (30 hours/week)
Average daily dose:	0 0	79 183	134 ppm (as if continuous) 311 mg/kg/day
Equivalent human dose:	0	37	62 mg/kg/day (surface-area adjusted)
Tumor incidence:	1/62	5/78	1/78 (alive at first tumor, 12+ months)
Tumor percentage:	2%	6%	1%
Statistical significance:	--	NS	NS
Trend significance:	NS	significant nonlinearity ($p<0.05$)	
Animal weight:	0.565 kg (control, at end of exposure period)		
Exposure period:	18.3 months		
Study length:	24 months		
Animal lifespan:	24 months		
Potency (q_1^*):	1E-03 (mg/kg/day)		

contrary this approach is consistent with the Agency's cancer guidelines. Exposures are converted to a mg/kg/day basis by assuming that the styrene vapor is an aerosol that is absorbed proportionately to the amount of air breathed in. Equivalent human exposures are calculated using a surface-area adjustment. The results of these calculations are summarized in Tables VIII-2 and VIII-3.

The potency is estimated using a linear nonthreshold model (the linearized multistage model with two stages), consistent with the Agency's cancer guidelines. From female rats the potency is estimated to be 2×10^{-3} per mg/kg/day continuous lifetime exposure to styrene. From male rats the potency is estimated to be 1×10^{-3} per mg/kg/day.

These estimates are not changed much by the alternative analyses possible at this time. One analysis considers the absence of a dose-response trend in the 1000 ppm groups to be indicative of high-dose saturation that is not relevant to low-dose exposures. Therefore the 1000 ppm groups are dropped and the one-stage model is used to fit the remaining data. Another analysis assumes that styrene is absorbed proportionately to the metabolic rate so that an exposure expressed in ppm for experimental animals is equivalent to the same concentration for humans without further adjustment for surface area. Under this analysis the potency is reduced by a factor of 2.6. Although the inhalation potency has been calculated according to the Agency's cancer guidelines (see Tables VIII-2 and VIII-3), some significant considerations limit the reliability and usefulness of this estimate. This study, however, does not show a dose-response trend.

Evidence that ingestion of styrene causes cancer in laboratory animals comes from two studies. Ponomarkov and Tomatis (1978) administered styrene by olive oil gavage to female O_{20} mice, C56Bl mice and BDIV rats once on the 17th day of gestation and then weekly throughout their offspring's lifetimes. A statistically significant increased incidence and earlier onset of lung tumors were observed in the O_{20} offspring, but the high background tumor rate (up to 67%) in this strain makes it unsuitable for a potency estimate. A few rare tumors were observed in the BDIV offspring, but the data are too sketchy for a reliable potency estimate. In the C57Bl offspring there was a statistically significant increased incidence of liver tumors compared with pooled untreated and vehicle controls. Details of this study are given in Table VIII-4.

NCI (1979) administered styrene at levels of 150 or 300 mg/kg daily by corn oil gavage to B6C3F1 mice. Exposure was terminated after 78 weeks, and the study was terminated after 91 weeks. Statistically significant increased incidences of lung alveolar/bronchiolar adenomas or carcinomas were observed in both exposed groups with a statistically significant dose-response trend. Details of this study are given in Table VIII-5.

To estimate the cancer potency the exposures are prorated over the animals' lifetimes. Equivalent human exposures are calculated using a surface-area adjustment. The results of these calculations are summarized in Tables VIII-4 and VIII-5.

The potency is estimated using a linear nonthreshold model (the linearized multistage model with one or two stages), consistent with the

TABLE VIII-4

Summary of Tumor Incidence in C57Bl Mice

Substance:	Styrene	
Reference:	Ponomarkov and Tomatis, 1978	
Sex, Strain, Species:	Male, C57Bl mouse	
Exposure route, Vehicle:	Oral by olive oil gavage	
Tumor site, Type:	Liver hepatocellular carcinoma	
Nominal dose:	0.0	300.0 mg/kg/day (once a week)
Average daily dose:	0.0	42.9 mg/kg/day
Equivalent human dose:	0.0	3.2 mg/kg/day (surface-area adjusted)
Tumor incidence:	0/59	3/24
Tumor percentage:	0%	13%
Statistical significance:	--	0.02
Trend significance:	<0.01	linearity OK
Animal weight:	0.03 kg (assumed)	
Exposure period:	120 weeks	
Study length:	120 weeks	
Animal lifespan:	120 weeks	
Potency (q_1^*):	9E-02 (mg/kg/day)	

TABLE VIII-5

Summary of Tumor Incidence of B6C3F1 Mice

Substance:	Styrene		
Reference:	NCI, 1979		
Sex, Strain, Species:	Male, B6C3F1 mouse		
Exposure route, Vehicle:	Oral by corn oil gavage		
Tumor site, Type:	Lung alveolar/bronchiolar adenoma/carcinoma		
Nominal dose:	0	150	300 mg/kg/day (5 days/week)
Average daily dose:	0	92	184 mg/kg/day
Equivalent human dose:	0	7	14 mg/kg/day (surface-area adjusted)
Tumor incidence:	0/20	6/44	9/43
Tumor percentage:	0%	14%	21%
Statistical significance:	--	0.09	0.02
Trend significance:	<0.02	linearity OK	
Animal weight:	0.03 kg (assumed)		
Exposure period:	78 weeks		
Study length:	91 weeks		
Animal lifespan:	91 weeks		
Potency (q_1^*):	3E-02 (mg/kg/day)		

Agency's cancer guidelines. From the C57Bl mice the potency is estimated to be 9×10^{-2} per mg/kg/day continuous lifetime exposure to styrene. From the B6C3F1 mice the potency is estimated to be 3×10^{-2} per mg/kg/day.

The negative results for carcinogenicity in BDIV rats (by gavage, Ponomarkov and Tomatis, 1978) and F344 rats (by gavage; NCI, 1979) may be more a reflection of inadequate dosing than evidence for actual noncarcinogenicity. In BDIV rats there were no signs of toxicity to indicate that an MTD was being approached and although the high-dose group in the NCI F344 rat bioassay showed severe early mortality, no signs were seen in the medium and low doses, thus calling into question the interpretive utility of the dose groups in this latter study. Given these considerations, the negative results are better thought of as nonpositive and do not negate, from an interpretive perspective, the positive carcinogenic evidence seen in the other bioassays.

Because the study with male B6C3F1 mice (NCI, 1979) included more than one exposure level and showed a dose-related trend, it is considered to provide a more reliable basis for estimating the potency. Using the linearized multistage model, 3×10^{-2}/(mg/kg/day) (slope factor, q_1^*) continuous lifetime exposure is considered at this time to be the cancer potency for styrene by ingestion.

All available information points to the importance of kinetic modeling in determining the effective dose in the animal studies and in extrapolating the results to low-dose human exposures. These analyses are currently in progress.

Existing Guidelines, Recommendations and Standards

The OSHA health standard for styrene exposure is an 8-hour TWA concentration of 100 ppm, a ceiling concentration of 200 ppm, and a maximum peak for ≤5 minutes in any 3-hour period of 600 ppm (29 CFR 1910.1000; Table Z-2). The ACGIH (1982) has established the TWA-TLV for styrene in workroom air as 50 ppm with a STEL of 100 ppm. The TLV was reduced from 100 ppm in 1981 (ACGIH, 1981). NIOSH (1983) recommended a styrene concentration limit in workplace air of 50 ppm TWA for up to a 10-hour day, 40-hour workweek and a ceiling concentration of 100 ppm determined during any 15-minute sampling period. More recently styrene levels in workroom air have been lowered to 20 ppm both in Finland and in the Federal Republic of Germany. The key factor in setting this standard was chromosomal aberrations found in styrene exposed workers (Vanio, 1987).

Special Groups at Risk

Individuals with occupational exposure to styrene may be regarded as a special group at risk because of their regular exposure to styrene rather than their intrinsic susceptibility. There is some indication that pregnant women and their fetuses may be more susceptible to styrene exposure. Styrene metabolism was inhibited by coexposure to toluene or trichloroethylene; synergistic or antagonistic effects of multiple pollutant exposures have not been further documented in the available literature (Ikeda et al., 1972; Ikeda and Hirayama, 1978).

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